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(54) **METHOD OF DETECTING *PAECILOMYCES VARIOTII***

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CPC **C12Q 1/6895** (2013.01); **C07K 14/37**
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(58) **Field of Classification Search**

None

See application file for complete search history.

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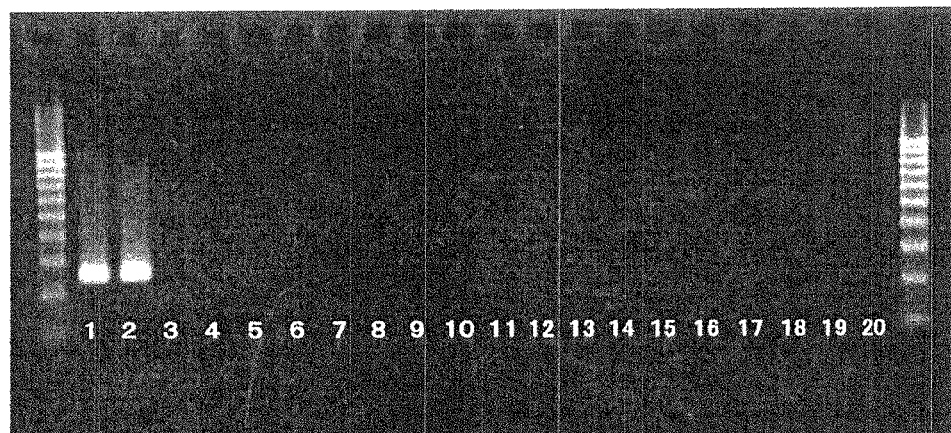
(57) **ABSTRACT**

A method of detecting *Paecilomyces variotii*, containing identifying *Paecilomyces variotii* using a nucleic acid represented by the following nucleotide sequence (a) or (b):

(a) a partial nucleotide sequence of a β -tubulin gene set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33, or a complementary sequence thereof; and

(b) a nucleotide sequence which includes a nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33 which has a deletion, substitution, or addition of one to several nucleotides and can be used for detecting *Paecilomyces variotii*, or a complementary sequence thereof.

12 Claims, 5 Drawing Sheets



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FIG. 1

Partial nucleotide sequence of β -tubulin gene of *Paecilomyces variotii* IFM40913 strain, and position recognized by the primers of primer set 1 of the present invention

```
1  TGGTAACCAA ATCGGTGCTG CTTTCTGGTA TGGTGCAAC CAGCAGGAGA AATGAAACAA AGAGCCTAGA GTCCGTTTGG 80
*****
< =====F3 =====>
81  GGACGTGGAA GGCTCAAGTG ATCAGAATTG GAGGTGCTAA CGATCCTATA GGCAGACCAT CTCTGGTGAG CACGGCCTTG 160
*****

===F2===== ==>
161  ACGGCTCTGG TGTGTAAGTA CACGATATCT GGGGATGCTT CGACACGGAA TCGAGAGAGA CGACTGACGA TGGATTAGCT 240
*****

<===== ==LF===== ==> <===== F1c===== ==>
<=====B 1c=====> <===== =LB===== ==>
241  ACAATGGCTC CTCGGACCTT CAGCTCGAGC GCATGAACGT CTACTTCAAC GAGGTAGTTG TTGACCCATAT GATCCCAAGA 320
*****

<=====B
321  GGAACGCTCC ATGAGCTCAC CAATAAATAG GCCGCTGGCA AGAAGTACGT TCCTCGTGCC GTCCTCGTCS ACCTCGAGCC 400
*****

2=====> <= =====B3= =====>
401  TGGTACCATG GACGCTGTCC GTGCTGGTCC TTTCGGCCAG CTCTTCGCCC CTGACAACTT CGTCTTCGGT CAGTCGGGTG 480
*****

481  CTGGTAACAA CTGGGCCAAG GGTCACTACA CTG 513
*****
```

* represents a nucleotide in the complementary strand.

FIG. 2

Partial nucleotide sequence of β -tubulin gene of each of *Paecilomyces variotii* IFM40913 and IFM40915 strains, and position recognized by the primers of primer set 2 of the present invention

```

P_variotii_40913    AGAGCCTGAGTCGGTTTGGGACGTCGAAGCCTCAAGTGATCAGATTTGGAGGTGCTAA 120
P_variotii_40915    AGAGCCTGAGTCGGTTTGGGACGTCGAAGCCTCAAGTGATCAGATTTGGAGGTGCTAA 120
*****

P_variotii_40913    CGATTCTATAGGCAGACCATCTCTGGTGAGCACGGCCTTGACGGCTCTGGTGTTAAGTA 180
P_variotii_40915    CGATTCTATAGGCAGACCATCTCTGGTGAGCACGGCCTTGACGGCTCTGGTGTTAAGTA 180
*****

<=====F3=====> <=====F2=====>
P_variotii_40913    CACGATATCTGGGGATGCTTCGACACGGAATCGAGAGACGACTGACGATGGATTAGCT 240
P_variotii_40915    CACGATATCTGGGGATGCTTCGACACGGAATCGAGAGACGACTGACGATGGATTAGCT 240
*****<=====

P_variotii_40913    ACAATGGCTCCTCCGAC---TTCAGCTCGAGCGCATGAAGTCTACTTCAACGAGGTAG 297
P_variotii_40915    ACAATGGCTCCTCCGACNACCTTCAGCTCGAGCGCATGAAGTCTACTTCAACGAGGTAG 300
*****

===LF=====> <=====F1c=====>
P_variotii_40913    TTGTTGACCCCTATGATCCCAAGAGGAACGGTCCATGAGCTCACCATAAATAGGCCGCTG 357
P_variotii_40915    TTGTTGACCCCTATGATCCCAAGAGGAACGGTCCATGAGCTCACCATAAATAGGCCGCTG 360
*****<=====

P_variotii_40913    GCAAGAAGTACGTTCTCGTGCCGTCCTCGTCGACCTCGAGCCTGGTACCATGGACGCTG 417
P_variotii_40915    GCAAGAAGTACGTTCTCGTGCCGTCCTCGTCGACCTCGAGCCTGGTACCATGGACGCTG 420
*****<=====B2=====> <=====B3=====>

```

* represents a nucleotide in the complementary strand.

FIG. 3

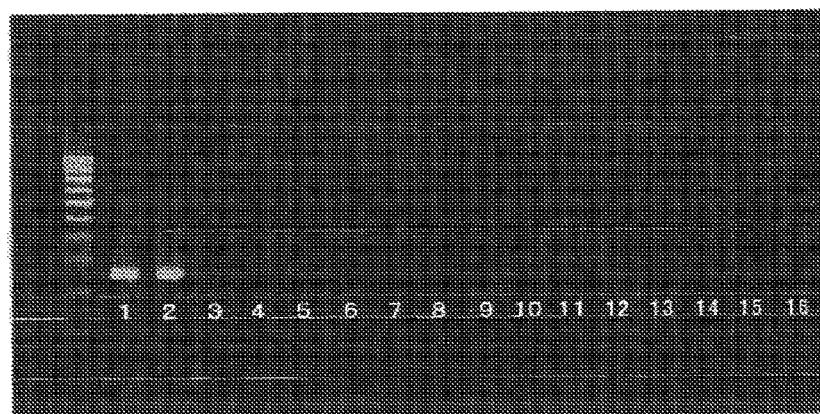


FIG. 4

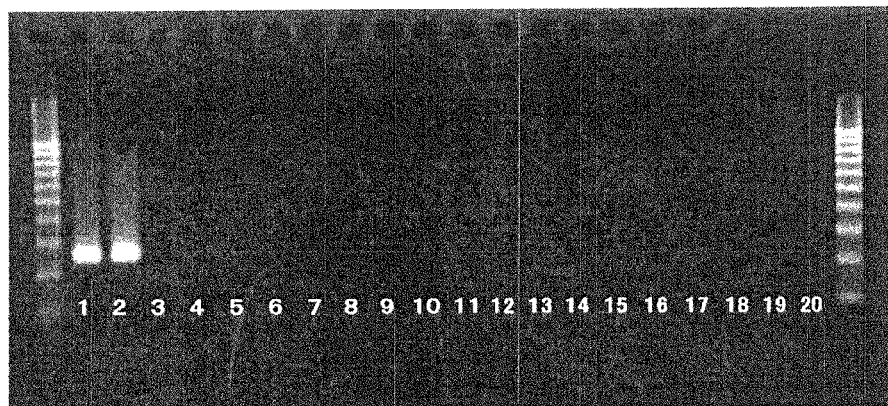


FIG. 5

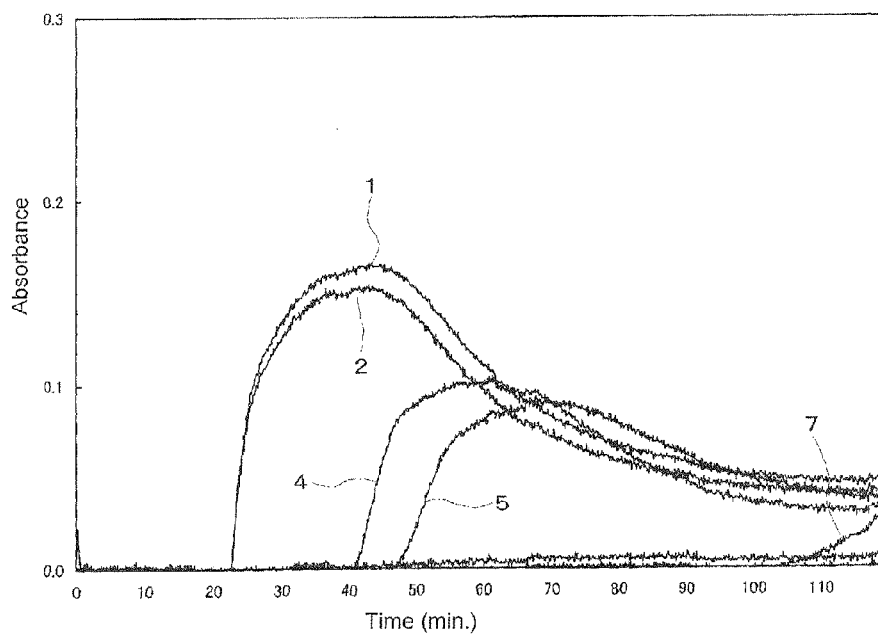


FIG. 6

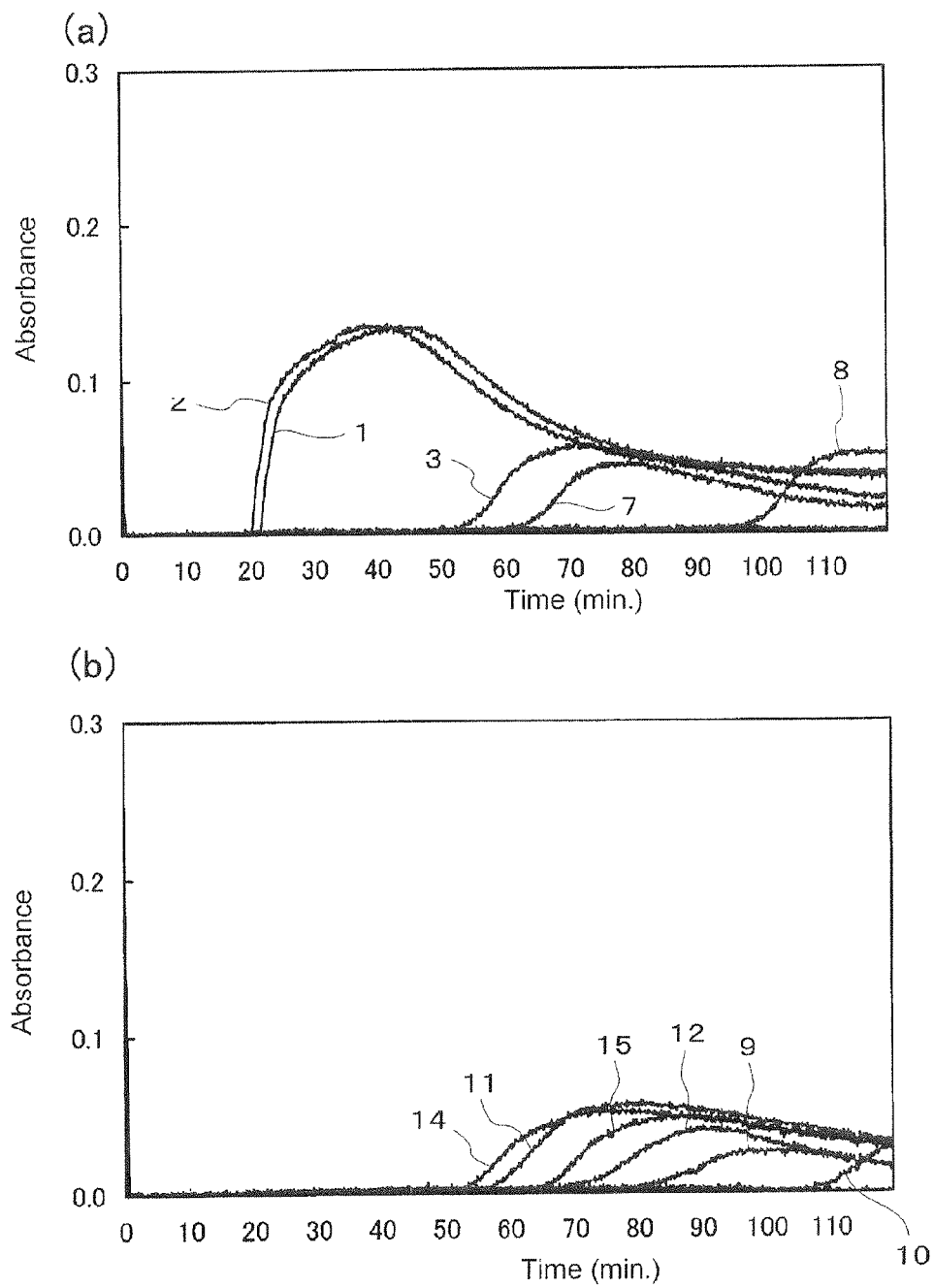
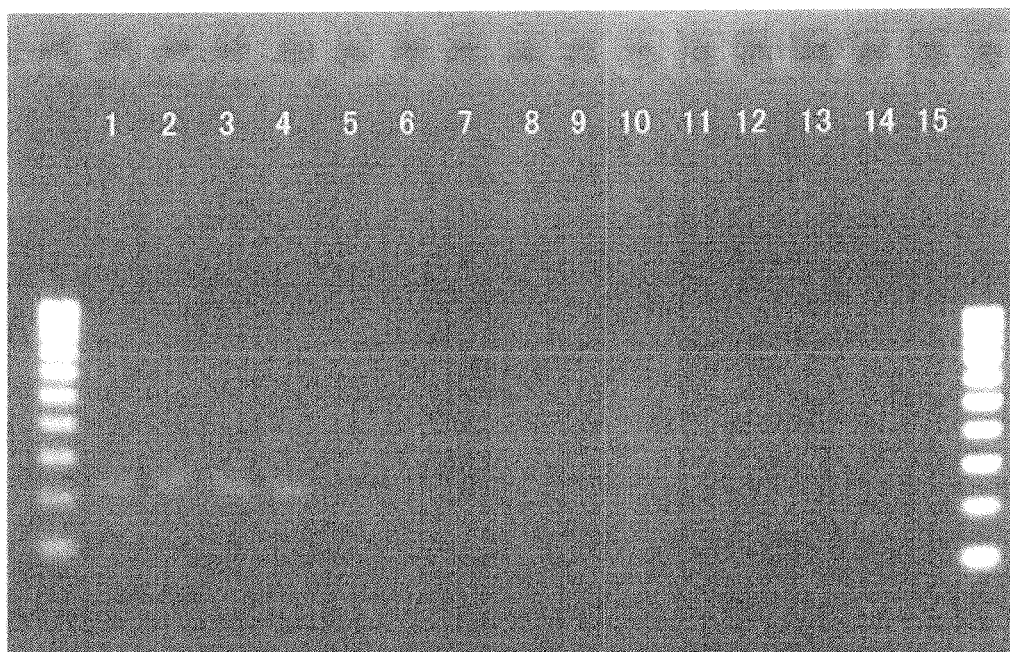


FIG. 7



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METHOD OF DETECTING *PAECILOMYCES* *VARIOTII*

REFERENCE TO SEQUENCE LISTING
SUBMITTED ELECTRONICALLY

The content of the electronically submitted substitute sequence listing, file name: 2537_0430000_sequencelisting_2ndSubstitute_ascii.txt, size: 17,083 bytes; and date of creation Jan. 20, 2015, filed herewith, is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present invention relates to a method of detecting *Paecilomyces Variotii*.

BACKGROUND ART

Paecilomyces variotii is an imperfect fungus which is widely distributed throughout nature. *Paecilomyces variotii* forms asexual spores including double cell walls called chlamydospores in its life cycle. The chlamydospores of *Paecilomyces variotii* are known to have very high resistance to stress, and to live and proliferate even under conditions for sterilizing general fungi by heat and a reagent, resulting in growth of mold. Therefore, *Paecilomyces variotii* is regarded as a harmful fungus in food industry and toiletry industry. Accordingly, in establishment of antiseptic and antifungal systems, detection and identification of *Paecilomyces variotii* is considered to be very important.

Detection and identification of *Paecilomyces variotii* is performed mainly by morphological classification through culture. In this method, it is necessary to continue the culture until morphological characters appear, and hence it takes a long period of time (at least 14 days) to perform the method. Moreover, the morphological identification requires a very high level of professionalism, and the identification results may vary depending on judges and have a problem in reliability. Therefore, it is required to establish a detection and identification method which solves the problems of rapidness and reliability.

As a method of rapidly and reliably detecting a fungus, an amplification method which targets a specific nucleotide sequence of a gene (such as the PCR method or the LAMP method) is known (see, for example, JP-T-11-505728 ("JP-T" means searched and published International patent publication), JP-A-2006-61152 ("JP-A" means unexamined published Japanese patent application), JP-A-2006-304763 and JP-A-2007-174903). However, a gene region specific to *Paecilomyces variotii* has not been clarified. Therefore, such method has a problem in that it is difficult to detect *Paecilomyces variotii* specifically and rapidly.

SUMMARY OF INVENTION

An object of the present invention is to provide a method with which *Paecilomyces variotii* can be detected specifically, easily, and rapidly, which is known to be a harmful fungus in food industry and toiletry industry. A further object of the present invention is to provide DNA, a primer set, an oligonucleotide and a detection kit, which can be applied to the method.

The difficulty in detection of *Paecilomyces variotii* as described above is caused by false positive and false negative results in the PCR method using known conventional primers. The inventors of the present invention considered that this

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problem is caused by difficulty in design of sensitive primers for specifically and rapidly detecting and discriminating *Paecilomyces variotii* because database of the genes of *Paecilomyces variotii* is now weak and the gene region specific to *Paecilomyces variotii*, conserved at species level, has not been clarified accurately.

In view of such problems, the inventors of the present invention have made extensive studies to search a novel DNA region capable of specifically detecting and discriminating *Paecilomyces variotii*. As a result, the inventors have found out that the β -tubulin gene of *Paecilomyces variotii* includes a region having a specific nucleotide sequence which can be clearly different from that of another fungus (hereinafter, also referred to as "variable region"). Moreover, the inventors have found out that such *Paecilomyces variotii* can be detected specifically and rapidly by targeting the variable region. The present invention has been completed based on the findings.

The present invention resides in a method of detecting *Paecilomyces variotii*, containing identifying *Paecilomyces variotii* using a nucleic acid represented by the following nucleotide sequence (a) or (b):

(a) a partial nucleotide sequence of a β -tubulin gene set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33, or a complementary sequence thereof; and

(b) a nucleotide sequence which includes a nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33 which has a deletion, substitution, or addition of one to several nucleotides and can be used for detecting *Paecilomyces variotii*, or a complementary sequence thereof.

Further, the present invention resides in a DNA represented by the nucleotide sequence (a) or (b) described above which is used for detecting *Paecilomyces variotii*.

Further, the present invention resides in an oligonucleotide for detecting *Paecilomyces variotii*, which can hybridize with a nucleic acid represented by the nucleotide sequence (a) or (b) described above and act as a nucleic acid probe or nucleic acid primer for specifically detecting *Paecilomyces variotii*.

Further, the present invention resides in an oligonucleotide pair or oligonucleotide group for detecting *Paecilomyces variotii*, containing at least one pair or one group selected from the group consisting of a pair of the following oligonucleotides (c) and (d), a pair of the following oligonucleotides (e) and (f), a pair of the following oligonucleotides (e) and (g), and a group of the following oligonucleotides (e), (f), and (g):

(c) an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 5, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 5 and can be used as a nucleic acid primer;

(d) an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 6, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 6 and can be used as a nucleic acid primer;

(e) an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 7, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 7 and can be used as a nucleic acid primer;

(f) an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 8, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 8 and can be used as a nucleic acid primer; and

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(g) an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 9, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 9 and can be used as a nucleic acid primer.

Further, the present invention relates to a kit for detecting *Paecilomyces variotii*, containing the above-described oligonucleotide pair or oligonucleotide group.

Further, the present invention relates to a primer set, containing:

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 10;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 11;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 12;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 13;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 14; and

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 15;

wherein the primer set is used for detecting *Paecilomyces variotii* by Loop mediated isothermal amplification (LAMP) method.

Further, the present invention relates to a primer set, containing:

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 10;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 11;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 12; and

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 13;

wherein the primer set is used for detecting *Paecilomyces variotii* by Loop mediated isothermal amplification (LAMP) method.

Further, the present invention relates to a primer set, containing:

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 16;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 17;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 18;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 19;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 20; and

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 21;

wherein the primer set is used for detecting *Paecilomyces variotii* by Loop mediated isothermal amplification (LAMP) method.

Further, the present invention relates to a primer set, containing:

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 16;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 17;

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 18; and

a primer consisting of the nucleotide sequence set forth in SEQ ID NO: 19;

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wherein the primer set is used for detecting *Paecilomyces variotii* by Loop mediated isothermal amplification (LAMP) method.

Further, the present invention relates to a kit for detecting *Paecilomyces variotii*, containing:

the above-described primer set for use in the LAMP method;

a DNA polymerase; and

dNTP containing dATP, dCTP, dGTP and dTTP.

According to the present invention, it is possible to provide a method with which *Paecilomyces variotii* can be detected specifically, easily, and rapidly, which is known to be a harmful fungus in food industry and toiletry industry. Further, according to the present invention, it is possible to provide DNA, a primer set, an oligonucleotide and a detection kit, which can be applied to the method.

BRIEF DESCRIPTION OF DRAWINGS

[FIG. 1] FIG. 1 is a diagram illustrating the position relationship of nucleotide sequences recognized by the primers of primer set 1 of the present invention in the nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM40913 strain SEQ ID NO:1.

[FIG. 2] FIG. 2 is a diagram illustrating the position relationship of nucleotide sequences recognized by the primers of primer set 2 of the present invention in the nucleotide sequence of the β -tubulin genes of *Paecilomyces variotii* IFM40913 strain and (bases 61-417 of SEQ ID NO: 1) and *Paecilomyces variotii* IFM40915 strain (bases 61-420 of SEQ ID NO: 2).

[FIG. 3] FIG. 3 is an electrophoretogram of PCR products obtained by using the oligonucleotides (c) and (d) of the present invention in Example 1.

[FIG. 4] FIG. 4 is an electrophoretogram of PCR products obtained by using the oligonucleotides (c) and (d) of the present invention in Example 1.

[FIG. 5] FIG. 5 is a graph illustrating the detection sensitivity of *Paecilomyces variotii* by real-time turbidity monitoring method in Example 2. The numeral 1 denotes the detection sensitivity of a sample including genomic DNA derived from *Paecilomyces variotii* IFM40913 strain; the numeral 2 denotes the detection sensitivity of a sample including genomic DNA derived from *Paecilomyces variotii* IFM40915 strain; the numeral 4 denotes the detection sensitivity of a sample including genomic DNA derived from *Byssoschlamys nivea* IFM51244 strain; the numeral 5 denotes the detection sensitivity of a sample including genomic DNA derived from *Hamigera avellanea* IFM42323 strain; and the numeral 7 denotes the detection sensitivity of a sample including genomic DNA derived from *Talaromyces luteus* IFM53242 strain.

[FIG. 6] FIG. 6(a) and FIG. 6(b) each area graph illustrating the detection sensitivity of *Paecilomyces variotii* by real-time turbidity monitoring method in Example 3. In FIG. 6(a), the numeral 1 denotes the detection sensitivity of a sample including genomic DNA derived from *Paecilomyces variotii* IFM40913 strain; the numeral 2 denotes the detection sensitivity of a sample including genomic DNA derived from *Paecilomyces variotii* IFM40915 strain; the numeral 3 denotes the detection sensitivity of a sample including genomic DNA derived from *Byssoschlamys fluva* IFM48421 strain; the numeral 7 denotes the detection sensitivity of a sample including genomic DNA derived from *Neosartorya fischeri* IFM46945 strain; and the numeral 8 denotes the detection sensitivity of a sample including genomic DNA derived from *Neosartorya spinosa* IFM46967 strain. In FIG. 6(b), the

numeral 9 denotes the detection sensitivity of a sample including genomic DNA derived from *Neosartorya glabra* IFM46949 strain; the numeral 10 denotes the detection sensitivity of a sample including genomic DNA derived from *Neosartorya hiratsukae* IFM47036 strain; the numeral 11 denotes the detection sensitivity of a sample including genomic DNA derived from *Alterraria alternata* IFM41348 strain; the numeral 12 denotes the detection sensitivity of a sample including genomic DNA derived from *Aureobasidium pullulans* IFM41409 strain; the numeral 14 denotes the detection sensitivity of a sample including genomic DNA derived from *Fusarium oxysporum* IFM50002 strain; and the numeral 15 denotes the detection sensitivity of a sample including genomic DNA derived from *Trichoderma viride* IFM40938 strain.

[FIG. 7] FIG. 7 is an electrophoretogram of PCR products obtained by using the oligonucleotides (e) to (g) of the present invention in Example 4.

MODE FOR CARRYING OUT THE INVENTION

Hereinafter, the present invention is described in detail.

The present invention is a method of specifically discriminating and detecting *Paecilomyces variotii* by identifying *Paecilomyces variotii* using a nucleic acid represented by a specific partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii*, i.e., a nucleic acid represented by a nucleotide sequence in a region (variable region) which is present in the β -tubulin gene sequence of *Paecilomyces variotii* and is specific to *Paecilomyces variotii*. In this description, the "variable region" is a region where nucleotide mutations tend to accumulate in the β -tubulin gene, and the nucleotide sequence in this region is significantly different from that of another fungus. The "*Paecilomyces variotii*" in the present invention is a filamentous deuteromycete and belongs to the genus *Paecilomyces*. Further, the " β -tubulin" is a protein which constitutes a microtubule, and the " β -tubulin gene" is a gene encoding β -tubulin.

In the method of detecting *Paecilomyces variotii* of the present invention, *Paecilomyces variotii* is identified using a nucleic acid represented by the following nucleotide sequence (a) or (b).

- (a) A partial nucleotide sequence of a β -tubulin gene set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33, or a complementary sequence thereof
- (b) A nucleotide sequence which includes a nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33 which has a deletion, substitution, or addition of one to several nucleotides and can be used for detecting *Paecilomyces variotii*, or a complementary sequence thereof.

The variable region of the β -tubulin gene represented by the nucleotide sequence (a) or (b) has a nucleotide sequence specific to *Paecilomyces variotii* and can be clearly different from that of another general fungus. Therefore, according to the method of detecting *Paecilomyces variotii* of the present invention, *Paecilomyces variotii* can be detected specifically and rapidly. Moreover, DNA represented by the nucleotide sequence (a) or (b) can be used for detecting *Paecilomyces variotii*.

Partial nucleotide sequences of the β -tubulin gene of *Paecilomyces variotii* are described based on SEQ ID NOS: 1 and 2. The nucleotide sequence set forth in SEQ ID NO: 1 is a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM40913 strain, and the nucleotide sequence set forth in SEQ ID NO: 2 is a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM40915 strain.

Moreover, parts of the partial nucleotide sequences of the β -tubulin gene of *Paecilomyces variotii* IFM40913 and IFM40915 strain set forth in SEQ ID NOS: 1 and 2 are compared and described in FIG. 2. The inventors of the present invention have found out that the nucleotide sequences in the region of the position 50 to 100 and the region of the position 280 to 340 in the nucleotide sequences set forth in SEQ ID NOS: 1 and 2 are particularly poorly conserved among the fungi, and each of the fungi species has a specific nucleotide sequence in these regions. The present invention targets these partial nucleotide sequences of the β -tubulin gene.

Moreover, partial nucleotide sequences of the β -tubulin gene of *Paecilomyces variotii* are described based on SEQ ID NOS: 3 and 4 and 22 to 33.

The nucleotide sequence set forth in SEQ ID NO: 3 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* EUO37073 strain.

The nucleotide sequence set forth in SEQ ID NO: 4 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM50293 strain.

The nucleotide sequence set forth in SEQ ID NO: 22 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* NBRC4855 strain.

The nucleotide sequence set forth in SEQ ID NO: 23 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM52147 strain.

The nucleotide sequence set forth in SEQ ID NO: 24 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM52145 strain.

The nucleotide sequence set forth in SEQ ID NO: 25 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM51028 strain.

The nucleotide sequence set forth in SEQ ID NO: 26 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM51195 strain.

The nucleotide sequence set forth in SEQ ID NO: 27 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM55619 strain.

The nucleotide sequence set forth in SEQ ID NO: 28 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* NBRC5479 strain.

The nucleotide sequence set forth in SEQ ID NO: 29 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM51027 strain.

The nucleotide sequence set forth in SEQ ID NO: 30 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* NBRC31967 strain.

The nucleotide sequence set forth in SEQ ID NO: 31 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* NBRC31685 strain.

The nucleotide sequence set forth in SEQ ID NO: 32 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* SUM3339 strain.

The nucleotide sequence set forth in SEQ ID NO: 33 represents a partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM50294 strain.

The inventors of the present invention have found out that the partial nucleotide sequences of the β -tubulin gene are also particularly poorly conserved among the fungi, and each of the fungi species has a specific nucleotide sequence. The present invention also targets the partial nucleotide sequences of the β -tubulin gene.

The nucleic acid represented by the partial nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* to be used in the detection method of the present invention (the nucleotide sequence in the variable region) is a nucleic acid

represented by the nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33 or the complementary sequence thereof.

The nucleotide sequences set forth in SEQ ID NOS: 1 to 4 and 22 to 33 or complementary sequences thereof are nucleotide sequences of the variable regions of the β -tubulin gene, which are isolated and identified from *Paecilomyces variotii* IFM40913, IFM40915, EUO37073, IFM50293, NBRC4855, IFM52147, IFM52145, IFM51028, IFM51195, IFM55619, NBRC5479, IFM51027, NBRC31967, NBRC31685, SUM3339 and IFM50294, respectively. These sequences have very high homology in *Paecilomyces variotii* and have low homology to a fungus other than *Paecilomyces variotii*, and hence it is possible to specifically distinguish and identify only *Paecilomyces variotii* by confirming whether a sample has such nucleotide sequence or not.

Also in the case of using nucleic acids represented by the nucleotide sequences set forth in SEQ ID NOS: 1 to 4 and 22 to 33 with a deletion, substitution, or addition of one to several nucleotides or complementary sequences thereof, it is possible to specifically distinguish and identify only *Paecilomyces variotii*.

(Hereinafter, the nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33 or a complementary sequence thereof, and the nucleotide sequence set forth in any one of SEQ ID NOS: 1 to 4 and 22 to 33 with a deletion, substitution, or addition of one to several nucleotides, which can be used for detecting *Paecilomyces variotii*, or a complementary sequence thereof are also referred to as "nucleotide sequences in variable regions of the β -tubulin gene of the present invention".)

The method of identifying *Paecilomyces variotii* using a nucleic acid represented by the nucleotide sequence in the variable region of the β -tubulin gene of the present invention is not particularly limited, and may be performed by a usual genetic engineering procedure such as a sequencing method, a hybridization method, a PCR method, or a LAMP method.

In the detection method of the present invention, in order to identify *Paecilomyces variotii* using the nucleic acid represented by the nucleotide sequence in the variable region of the β -tubulin gene of the present invention, the nucleotide sequence of the β -tubulin gene in a sample is preferably determined to confirm whether the nucleotide sequence in the gene includes the nucleotide sequence of the nucleic acid (a) or (b) or not. That is, the detection method of the present invention includes: analyzing and determining the nucleotide sequence of the β -tubulin gene in a sample; comparing the determined nucleotide sequence with the nucleotide sequence in the variable region of the β -tubulin gene of the present invention; and identifying *Paecilomyces variotii* based on the matching or difference.

The method of analyzing and determining the nucleotide sequence is not particularly limited, and usual RNA or DNA sequencing means may be used.

Specific examples of the method include an electrophoresis method such as a Maxam-Gilbert method or a Sanger method, mass spectrometry, and a hybridization method. Examples of the Sanger method include a method of labeling a primer or terminator by a radiation labeling method, a fluorescent labeling method, or the like.

In the present invention, in order to identify and detect *Paecilomyces variotii* using the nucleic acid represented by the nucleotide sequence in the variable region of the β -tubulin gene of the present invention, an oligonucleotide for detection which can hybridize with the nucleic acid represented by the nucleotide sequence in the variable region of the β -tubulin

gene of the present invention and acts as a nucleic acid probe or a nucleic acid primer may be used.

The oligonucleotide for detection of the present invention may be one which can be used for detecting *Paecilomyces variotii*. That is, the oligonucleotide may be one which can be used as a nucleic acid primer or a nucleic acid probe for detection of *Paecilomyces variotii*, or one which can hybridize with the β -tubulin gene of *Paecilomyces variotii* under stringent conditions. It should be noted that, in this description, the "stringent conditions" includes, for example, the method described in Molecular Cloning-A LABORATORY MANUAL THIRD EDITION [Joseph Sambrook, David W. Russell. Cold Spring Harbor Laboratory Press], and examples thereof include conditions where hybridization is performed by incubating a solution containing 6 \times SSC (composition of 1 \times SSC: 0.15M sodium chloride, 0.015M sodium citrate, pH7.0), 0.5% SDS, 5 \times Denhardt and 100 mg/mL herring sperm DNA together with a probe at 65 $^{\circ}$ C. for 8 to 16 hours.

The oligonucleotide for detection of the present invention is more preferably an oligonucleotide represented by the nucleotide sequence which is in a region in the nucleic acid represented by the nucleotide sequence (a) or (b) and is set forth in any one of SEQ ID NOS: 5 to 21, or a complementary sequence thereof. Further, the oligonucleotide for detection of the present invention may be an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in any one of SEQ ID NOS: 5 to 21 or a complementary sequence thereof, and the homology is more preferably 80% or more, still more preferably 85% or more, even more preferably 90% or more, particularly preferably 95% or more. Further, the oligonucleotide for detection which can be used in the present invention includes an oligonucleotide represented by a nucleotide sequence set forth in any one of SEQ ID NOS: 5 to 21 or a complementary sequence with a mutation or modification such as a deletion, insertion or substitution of one or several, preferably one to five, more preferably one to four, still more preferably one to three, even more preferably one to two, particularly preferably one nucleotide. Moreover, an appropriate nucleotide sequence may be added to the nucleotide sequence set forth in any one of SEQ ID NOS: 5 to 21 or a complementary sequence thereof.

The nucleotide sequence homology is calculated, for example, by Lipman-Pearson method (Science, 227, 1435, (1985)). Specifically, it can be calculated by performing analysis using a homology analysis (Search homology) program of genetic information processing software Genetyx-Win (Software Development) while the unit size to compare (ktup) parameter is set to 2.

The bonding pattern of the oligonucleotide for detection includes not only a phosphodiester bond present in a natural nucleic acid but also a phosphoramidate bond and a phosphorothioate bond, for example.

The oligonucleotide for detection of the present invention may be used as a nucleic acid primer and a nucleic acid probe. The nucleic acid probe can be prepared by labeling the above-mentioned oligonucleotide with a labeling substance. The labeling substance is not particularly limited and may be a usual labeling substance such as a radioactive substance, an enzyme, a fluorescent substance, a luminescent substance, an antigen, a hapten, an enzyme substrate, or an insoluble carrier. The oligonucleotide may be labeled at its terminal or at the sequence other than the terminals, or at the sugar, phosphate group, or base moiety. Examples of means for detecting the label include: autoradiography in the case of a nucleic acid probe labeled with a radioisotope; a fluorescent microscope

in the case of a nucleic acid probe labeled with a fluorescent substance; and an analysis using a sensitive film or a digital analysis using a CCD camera in the case of a nucleic acid probe labeled with a chemiluminescent substance.

Further, the oligonucleotide may be bound to a solid-phase carrier and used as a capture probe. In this case, the capture probe and labeled nucleic acid probe may be combined and used in a sandwich assay, or a target nucleic acid may be labeled and captured.

In the detection method of the present invention, in order to identify and detect *Paecilomyces variotii*, hybridization is performed using, as a nucleic acid probe, preferably an oligonucleotide represented by the nucleotide sequence set forth in any one of SEQ ID NOS: 5 to 21 or the complementary sequence thereof, or an oligonucleotide which has 70% or more homology to the nucleotide sequence set forth in any one of SEQ ID NOS: 5 to 21 or the complementary sequence thereof and can be used as an oligonucleotide for detection, more preferably the oligonucleotide represented by the nucleotide sequence set forth in any one of SEQ ID NOS: 5 to 21.

In order to detect *Paecilomyces variotii* in a sample, an oligonucleotide represented by the nucleotide sequence set forth in any one of SEQ ID NOS: 5 to 21 or the complementary sequence thereof, or an oligonucleotide which has 70% or more homology to the nucleotide sequence set forth in any one of SEQ ID NOS: 5 to 21 or the complementary sequence thereof and can be used as an oligonucleotide for detection is labeled to prepare a nucleic acid probe, and the resultant nucleic acid probe is hybridized with DNA or RNA, followed by detection of the label of the hybridized probe by an appropriate detection method. The nucleic acid probe is hybridized specifically with part of the variable region of the β -tubulin gene of *Paecilomyces variotii*, and hence *Paecilomyces variotii* in a sample can be detected rapidly and easily. As a method of measuring the label of the nucleic acid probe hybridized with DNA or RNA, a usual method (such as a FISH method, a dot-blot method, a Southern-blot method, or a Northern-blot method) may be used.

Further, in the detection method of the present invention, in order to identify *Paecilomyces variotii* using the nucleic acid represented by the nucleotide sequence in the variable region of the β -tubulin gene of the present invention, a DNA fragment including the whole or a partial region of the nucleotide sequence is preferably amplified to confirm whether the amplified product is present or not. The method of amplifying the DNA fragment including the region is not particularly limited, and a usual method such as PCR (polymerase chain reaction) method, LCR (ligase chain reaction) method, SDA (strand displacement amplification) method, NASBA (nucleic acid sequence-based amplification) method, RCA (rolling-circle amplification) method, or LAMP (loop mediated isothermal amplification) method may be used. However, in the present invention, the PCR method or the LAMP method is preferably used.

A case where *Paecilomyces variotii* is detected by performing an amplification reaction by the PCR method in the present invention is described.

A primer consisting of the oligonucleotide for detection of the present invention is preferably an oligonucleotide which can hybridize with a region which is selected from the nucleotide sequence in the variable region of the β -tubulin gene of the present invention and satisfies the following four conditions:

- (1) the region contains a nucleotide sequence including about 10 continuous nucleotides in the nucleotide sequence of the gene specific to *Paecilomyces variotii*;

- (2) the oligonucleotide has a GC content of about 30% to 80%;
- (3) the oligonucleotide has low possibility to cause self-annealing; and
- (4) the oligonucleotide has a melting temperature (T_m value) of about 55° C. to 65° C.

In the (1) above, the “region contains a nucleotide sequence including about 10 continuous nucleotides in the nucleotide sequence of the gene specific to *Paecilomyces variotii* is present” refers to a region where the nucleotide sequences of different fungi species are particularly poorly conserved (that is, the region has particularly high specificity to *Paecilomyces variotii*) in the variable region of the β -tubulin gene of the present invention and where a nucleotide sequence including about 10 continuous nucleotides in the nucleotide sequence specific to *Paecilomyces variotii* is present. Moreover, in the (3) above, the “oligonucleotide has low possibility to cause self-annealing” means that the primers are expected not to bind to each other due to the nucleotide sequences of the primers.

The number of nucleotides in the oligonucleotide for detection of the present invention is not particularly limited, and is preferably 13 to 30, more preferably 18 to 23. The T_m value of the oligonucleotide in hybridization is preferably in a range of 55° C. to 65° C., more preferably 59° C. to 62° C. The GC content in the oligonucleotide is preferably 30% to 80%, more preferably 45% to 65%, most preferably about 55%.

Further, in the case of identification of *Paecilomyces variotii* using a nucleic acid represented by a partial nucleotide sequence of the β -tubulin gene set forth in SEQ ID NO: 1 or 2 or a complementary sequence thereof, or a nucleic acid represented by the nucleotide sequence which has a deletion, substitution, or addition of one or several nucleotides and can be used for detecting *Paecilomyces variotii*, or a complementary sequence thereof, the whole or part of the nucleotide sequence which is a part of the nucleotide sequences set forth in SEQ ID NOS: 1 and 2 and is located at the position 50 to 100 and/or the position 280 to 340 of the nucleotide sequence, or the whole or part of the nucleotide sequence with a deletion, substitution, or addition of one or several nucleotides is preferably amplified. In this case, the number of nucleotides in the oligonucleotide is not particularly limited, and the number is preferably 13 to 30, more preferably 18 to 23.

In order to detect *Paecilomyces variotii* by performing an amplification reaction by the PCR method, the following oligonucleotide (c) or oligonucleotide (d) are preferably used as nucleic acid primers, and the oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 5 and the oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 6 are more preferably used.

- (c) An oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 5, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 5 and can be used as a nucleic acid primer
- (d) An oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 6, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 6 and can be used as a nucleic acid primer

Further, the oligonucleotide pair for detecting *Paecilomyces variotii* of the present invention is a pair of oligonucleotides consisting of the oligonucleotide (c) and the oligonucleotide (d).

The oligonucleotides of SEQ ID NO: 5 and SEQ ID NO: 6 are present in the β -tubulin gene region of *Paecilomyces*

variotii and have nucleotide sequences identical to a partial nucleotide sequence of the variable region or complementary sequences thereof. These oligonucleotides can hybridize specifically with part of the DNA of *Paecilomyces variotii*.

The oligonucleotides (c) and (d) correspond to the region of the position 64 to 85 and the region of the position 306 to 325, respectively, in the nucleotide sequence set forth in SEQ ID NO: 1. Therefore, when the oligonucleotides are hybridized with the β -tubulin gene of *Paecilomyces variotii*, *Paecilomyces variotii* can be specifically detected.

Further, in the present invention, in order to detect *Paecilomyces variotii* by performing an amplification reaction by the PCR method, the following oligonucleotide (e), and the oligonucleotide (f) and/or oligonucleotide (g) are preferably used as nucleic acid primers; the oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 7, and the oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 8 and/or the oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 9 are more preferably used; and the oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 7, the oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 8 and the oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 9 are particularly preferably used.

(e) An oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 7, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 7 and can be used as a nucleic acid primer;

(f) An oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 8, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 8 and can be used as a nucleic acid primer; and

(g) An oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 9, or an oligonucleotide represented by a nucleotide sequence which has 70% or more homology to the nucleotide sequence set forth in SEQ ID NO: 9 and can be used as a nucleic acid primer.

Further, the oligonucleotide pair (oligonucleotide group) for detecting *Paecilomyces variotii* of the present invention is a pair of oligonucleotides consisting of the oligonucleotide (e) and the oligonucleotide (f), a pair of oligonucleotides consisting of the oligonucleotide (e) and the oligonucleotide (g), or a group of oligonucleotides consisting of the oligonucleotide (e), the oligonucleotide (f) and the oligonucleotide (g).

The oligonucleotides of SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9 are oligonucleotides having nucleotide sequences which are present in the β -tubulin gene region of *Paecilomyces variotii* and have nucleotide sequences identical to a partial nucleotide sequence of the variable region or complementary sequences thereof. These oligonucleotides can hybridize specifically with part of DNA of *Paecilomyces variotii*.

The oligonucleotides (e) and (f) correspond to the region of the position 98 to 116 and the region of the position 266 to 285, respectively, in the nucleotide sequence set forth in SEQ ID NO: 22. The oligonucleotides (e) and (g) correspond to the region of the position 92 to 110 and the region of the position 261 to 280, respectively, in the nucleotide sequence set forth in SEQ ID NO: 28.

Therefore, when the oligonucleotides are hybridized with the β -tubulin gene of *Paecilomyces variotii*, *Paecilomyces variotii* can be specifically detected.

Conditions of the PCR reaction in the present invention are not particularly limited as long as a DNA fragment of interest can be amplified to a detectable degree. A preferred example of the PCR reaction conditions is as follows. A cycle including: a thermal denaturation reaction for denaturation of double-stranded DNA into single strands at 95 to 98° C. for 10 to 60 seconds; an annealing reaction for hybridization of a primer pair with the single-stranded DNA at about 59° C. for about 60 seconds; and an elongation reaction for a reaction of a DNA polymerase at about 72° C. for about 60 seconds is repeated about 30 to 35 times.

In the present invention, confirmation of gene fragments amplified by the PCR method can be performed by a usual method. Examples of the method include, but not limited to, a method of integrating a nucleotide labeled with a radioactive substance or the like in an amplification reaction, a method including performing electrophoresis for PCR reaction products and confirming the existence of a band corresponding to the size of the amplified gene, a method of determining the nucleotide sequences of PCR reaction products, and a method of integrating a fluorescent substance into between the double strands of amplified DNA. In the present invention, the method including performing electrophoresis after a gene amplification treatment and confirming the existence of a band corresponding to the size of the amplified gene is preferred.

In the case where a sample contains *Paecilomyces variotii*, amplification of DNA fragments specific to the fungus can be observed by performing a PCR reaction using the oligonucleotide pair of the present invention as a primer set and performing electrophoresis for the resultant PCR reaction products.

Specifically, in the case of using the oligonucleotides (c) and (d) as nucleic acid primers, amplification of a DNA fragment of about 250 bp is observed; while in the case of using the oligonucleotide (e), and the oligonucleotide (f) and/or the oligonucleotide (g) as nucleic acid primers, amplification of a DNA fragment of about 200 bp is observed. The procedure can confirm whether the sample contains *Paecilomyces variotii* or not.

In the present invention, in the case where a DNA fragment including the variable region is amplified by the LAMP method, an isothermal complementary strand synthesis reaction can be performed because periodic temperature control is not required. Therefore, a specific fungus in a sample can be detected easily and rapidly.

The LAMP method is a loop-mediated isothermal amplification method which does not require the periodic temperature control essential for the PCR method (WO 00/28082 A1), which allows isothermal complementary strand synthesis reactions by annealing the 3'-end side of a primer to a nucleotide serving as a template to prepare a starting point of complementary strand synthesis and combining a primer that anneals to a loop formed at this time. In the LAMP method, at least four primers which recognize six nucleotide sequence regions of the nucleic acid serving as a template are required. The primers are designed so that the 3'-end side is certainly annealed to the nucleotide serving as a template, and hence a checking mechanism based on complementary binding of the nucleotide sequences functions repeatedly, which enables a sensitive and specific nucleic acid amplification reaction.

Six nucleotide sequence regions recognized by primers to be used in the LAMP method are referred to as F3, F2 and F1 in this order from the 5'-end side of a nucleotide serving as a template, and B3c, B2c and B1c in this order from the 3'-end side. Complementary sequences of F1, F2 and F3 are called

F1c, F2c and F3c, respectively. Complementary sequences of B1c, B2c and B3c are called B1, B2 and B3, respectively.

While the six nucleotide sequence regions may be selected by the following procedure, the present invention is not limited thereto.

Alignment of a nucleotide sequence of a gene of a fungus of interest is performed, and a plurality of primers are designed using software such as Primer Explorer V4 (HP of Eiken Chemical Co., Ltd.). The primers are synthesized and used in actual LAMP reactions, and primers which can specifically detect *Paecilomyces variotii* are adopted.

The details are as follows.

1. An alignment file of nucleotide sequence information of a target region of a fungus of interest is created using alignment software such as Clustal X.
2. Primers are designed based on the information of the alignment file using Primer Explorer V4 (HP of Eiken Chemical Co., Ltd.).
3. A set of primers which are more stable (which hardly have a dimer structure) and include many mutations on the edge of the elongation direction (species-specific mutations) is selected from designed primer set candidates. In particular, an inner primer including many mutations in the elongation direction increases the probability of differentiation from related species. In the case where primer set candidates cannot be created, design is performed so that the scope of the T_m value or the number of nucleotides in a primer is expanded.
4. A test is actually performed using the selected primer set to confirm the effectiveness. After confirmation of the effectiveness, loop primers are designed to shorten the reaction time.

Primers to be used in the LAMP method are designed by first determining the above-mentioned six nucleotide sequence regions from the nucleotide sequence of the target region, and then designing inner primers F and B and outer primers F and B to be described below.

The inner primer used in the LAMP method is an oligonucleotide having, at the 3' end, a nucleotide sequence that recognizes a certain nucleotide sequence region in a target nucleotide sequence and provides a synthesis origin; and having, at the 5' end, a nucleotide sequence complementary to an arbitrary region of a nucleic acid synthesis reaction product obtained with this primer at the origin. In this context, a primer containing a "nucleotide sequence selected from F2" at the 3' end and a "nucleotide sequence selected from F1c" at the 5' end is called an inner primer F (hereinafter, abbreviated to FIP), and a primer containing a "nucleotide sequence selected from B2" at the 3' end and a "nucleotide sequence selected from B1c" at the 5' end is called an inner primer B (hereinafter, abbreviated to BIP). The inner primers may have arbitrary nucleotide sequences including 0 to 50 nucleotides at a position between the F2 region and the F1c region or between the B2 region and the B1c region.

On the other hand, the "outer primer" is an oligonucleotide having a nucleotide sequence that recognizes a "certain nucleotide sequence region present on the 5'-end side of a certain nucleotide sequence region" (e.g., the above-mentioned F2 region or B2 region) in the target nucleotide sequence and provides a synthesis origin. Examples thereof include a primer including a nucleotide sequence selected from the F3 region and a primer including a nucleotide sequence selected from the B3 region. In this context, a primer containing a "nucleotide sequence selected from F3" is called an outer primer F (hereinafter, abbreviated to F3),

and a primer containing a "nucleotide sequence selected from B3" is called an outer primer B (hereinafter, abbreviated to B3).

In this context, "F" in each primer indicates that the primer complementarily binds to the anti-sense strand of the target nucleotide sequence and provides a synthesis origin. On the other hand, "B" in each primer indicates that the primer complementarily binds to the sense strand of the target nucleotide sequence and provides a synthesis origin.

In the amplification of the nucleic acid by the LAMP method, a loop primer(s) (hereinafter, abbreviated to LF and LB) can be preferably used in addition to the inner and outer primers. The loop primers refer to 2 primers (one for each of strands composing a double-strand) containing, at the 3' end, a nucleotide sequence complementary to a sequence in a loop formed by the annealing of complementary sequences present at the same strand of an amplification product obtained by the LAMP method. That is, the loop primer is a primer having a nucleotide sequence complementary to a nucleotide sequence of a single strand moiety of the loop structure on the 5'-end side in the dumbbell-like structure. The use of the loop primers increases nucleic acid synthesis origins in number and achieves reduction in reaction time and enhancement in detection sensitivity (WO 02/24902 Pamphlet).

The nucleotide sequence of the loop primer may be selected from nucleotide sequences in the target region or complementary strands thereof as long as the sequence is complementary to the nucleotide sequence of the single strand moiety of the loop structure on the 5'-end side in the dumbbell-like structure, or may be another nucleotide sequence. Further, one type or two types of loop primers may be used.

When a DNA fragment including the target region is amplified using at least four or more types of the above-mentioned primers, the DNA fragment can be amplified to an amount sufficient for specific and efficient detection of the DNA fragment. Therefore, a specific fungus can be detected by confirming whether the amplified product is present or not.

The primer which can be used for the LAMP method preferably includes 15 or more nucleotides, more preferably 20 or more nucleotides. Further, each primer may be an oligonucleotide of single nucleotide sequence or a mixture of oligonucleotides of a plurality of nucleotide sequences.

Further, the outer primer which can be used for the LAMP method may be used in the PCR method for amplifying a DNA fragment including a target region. In the PCR method, a DNA fragment of interest can be amplified by PCR using the above-mentioned primers, the β -tubulin gene in a sample as a template, and a heat-stable DNA polymerase.

Hereinafter, primer sets which are preferably used in detection of *Paecilomyces variotii* by the LAMP method are described. To specifically detect *Paecilomyces variotii*, the following primer set (primer set 1) is more preferably used. Primer Set 1 for Detecting *Paecilomyces variotii*

LPae1F3 primer: (SEQ ID NO: 10)
ACGATCCTATAGGCAGACCA
LPae1B3 primer: (SEQ ID NO: 11)
CCAGCGGCCTATTATTGGT
LPae1FIP primer: (SEQ ID NO: 12)
CGTCTCTCTCGATTCCGTGTCGCTTGACGGCTCTGGTGT

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-continued

LPae1BIP primer: (SEQ ID NO: 13)
CTCCGACCTTCAGCTCGAGCGGAGCGTTCCTCTGGGAT

To detect *Paecilomyces variotii*, loop primers are preferably used in addition to the above-mentioned primers. The following primers are preferably used as the loop primers. Loop Primers for Detecting *Paecilomyces variotii*

(SEQ ID NO: 14)
LPae1LF loop primer: TCCCCAGATATCGTGTACTTAC

(SEQ ID NO: 15)
LPae1LB loop primer: ACTTCAACGAGGTAGTTGTTG

FIG. 1 illustrates the position relationship of nucleotide sequences recognized by the above-mentioned primers in the nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM40913 strain.

In the present invention, the following primer set (primer set 2) is also preferably used in detection of *Paecilomyces variotii* by the LAMP method.

Primer Set 2 for Detecting *Paecilomyces variotii*

LPae2F3 primer: (SEQ ID NO: 16)
CGATATCTGGGGATGCTTCG

LPae2B3 primer: (SEQ ID NO: 17)
CGTCCATGGTACCAGGCT

LPae2FIP primer: (SEQ ID NO: 18)
ATGCGCTCGAGCTGAAGGTCCGGAATCGAGAGAGACGACT

LPae2BIP primer: (SEQ ID NO: 19)
TGATCCCAAGAGGAACGCCCCACGAGGAACGTACTTCTTGCC

To detect *Paecilomyces variotii*, loop primers are preferably used in addition to the above-mentioned primers. The following primers are preferably used as the loop primers. Loop Primers for Detecting *Paecilomyces variotii*

(SEQ ID NO: 20)
LPae2LF loop primer: GGAGGAGCCATTGTAGCTAA

(SEQ ID NO: 21)
LPae2LB loop primer: GAGCTCACCAATAAATAGGCC

FIG. 2 illustrates the position relationship of nucleotide sequences recognized by the above-mentioned primers in the nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* IFM40913 strain and *Paecilomyces variotii* IFM40915 strain.

The primer set for detecting *Paecilomyces variotii* of the present invention is a primer set to be used for detection by the LAMP method and includes a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 10, a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 11, a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 12 and a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 13; and the primer set preferably further includes primers consisting of oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NO: 14 and SEQ ID NO: 15.

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The another primer set for detecting *Paecilomyces variotii* of the present invention is a primer set to be used for detection by the LAMP method and includes a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 16, a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 17, a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 18 and a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 19; and the primer set preferably further includes primers consisting of oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NO: 20 and SEQ ID NO: 21.

When the above-described primer set is used, a DNA fragment including a target region of the β -tubulin gene of *Paecilomyces variotii* can be amplified specifically, rapidly, and sensitively by the LAMP method. Therefore, the existence of *Paecilomyces variotii* in a sample can be determined by confirming amplification of the DNA fragment.

Further, the oligonucleotide for detecting *Paecilomyces variotii* of the present invention is preferably an oligonucleotide represented by a nucleotide sequence corresponding to any one of the following (a') to (f') when nucleotide sequence regions F3, F2 and F1 are selected from the 5'-end side in a target region selected from the nucleotide sequence of the β -tubulin gene, nucleotide sequence regions B3c, B2c and B1c are selected from the 3'-end side in the target region, complementary nucleotide sequences of the B3c, B2c and B1c are called B3, B2 and B1, respectively, and complementary nucleotide sequences of the F3, F2 and F1 are called F3c, F2c and B1c, respectively.

(a') A nucleotide sequence having the sequence identical to that of the B2 region at the 3' terminal side and the sequence identical to that of the B1c region at the 5' terminal side
(b') A nucleotide sequence having the sequence identical to that of the B3 region
(c') A nucleotide sequence having the sequence identical to that of the F2 region at the 3' terminal side and the sequence identical to that of the F1c region at the 5' terminal side
(d') A nucleotide sequence having the sequence identical to that of the F3 region
(e') A nucleotide sequence having a sequence complement to a part between the B1 region and the B2 region
(f') A nucleotide sequence having a sequence complement to a part between the F1 region and the F2 region

The oligonucleotide of the present invention may be used not only as a primer for the LAMP method but also as, for example, a primer for the PCR method or a probe for detecting a nucleic acid.

An enzyme used in amplification of the DNA fragment including the target region by LAMP method is not particularly limited as long as it is generally used, and it is preferably a template-dependent nucleic acid synthetase having strand displacement activities. Such an enzyme includes Bst DNA polymerase (large fragment), Boa (exo-) DNA polymerase, and the Klenow fragment of *E. coli* DNA polymerase I; and preferably includes Bst DNA polymerase (large fragment). The enzyme that can be used in the present invention may be purified from viruses, bacteria, or the like or may be prepared by a gene recombination technique. These enzymes may be modified by fragmentation, amino acid substitution, or the like.

The temperature for amplification of the DNA fragment including the target region by the LAMP method is not particularly limited but is preferably 55 to 68° C., more preferably 60 to 65° C.

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The amplification of the DNA fragment including the target region can be confirmed by general method. For example, in the case of amplifying the DNA fragment including the target region by the LAMP method, the nucleic acid amplification products can be detected by hybridization of a labeled oligonucleotide specifically recognizing amplified nucleotide sequences or a fluorescent intercalator method (JP-A-2001-242169), or can be detected by directly applying the reaction solution after the completion of reaction to agarose gel electrophoresis. In the agarose gel electrophoresis, the LAMP amplification products are detected in the form of a ladder of many bands differing in base length.

Moreover, in the LAMP method, substrates are consumed in large amounts by nucleic acid synthesis, and pyrophosphoric acid ions as by-products are converted into magnesium pyrophosphate through its reaction with coexisting magnesium ions and makes the reaction solution cloudy to the extent that can be observed visually. Thus, the nucleic acid amplification reaction may be detected by confirming this cloudiness by use of a measurement apparatus that can optically observe time-dependent rises in turbidity after the completion of reaction or during reaction, for example, by confirming changes in absorbance at 400 nm by use of a usual spectrophotometer (WO 01/83817 Pamphlet).

In the present invention, primers to be used for amplifying the DNA fragment including the target region may be chemically synthesized based on designed sequences or purchased from a manufacturer of reagents. Specifically, the primers may be synthesized using an oligonucleotide synthesizer or the like. Moreover, after synthesis, the oligonucleotides may be purified by an adsorption column, high-performance liquid chromatography, or electrophoresis before use. Furthermore, an oligonucleotide having a nucleotide sequence with a substitution, deletion, insertion, or addition of one or several nucleotides may be synthesized by a known method.

The sample to be used in the present invention is not particularly limited and may be a food or drink itself, a raw material of the food or drink, an isolated fungus, a cultured fungus, or the like.

A method of preparing DNA from a sample is not particularly limited as long as DNA can be obtained at a sufficient purification degree and in a sufficient amount for detecting *Paecilomyces variotii*. While the sample may be used without purification, the sample may be subjected to a pre-treatment such as separation, extraction, concentration, or purification before use. For example, the sample may be purified by phenol and chloroform extraction or using a commercially available extraction kit to increase the purity of the nucleic acid before use. Moreover, DNA obtained by reverse transcription of RNA in a sample may be used.

A variety of reagents necessary for amplification of the DNA fragment including the target region using the primer of the present invention may be packaged as a kit in advance.

For example, the kit of the present invention includes the above-mentioned primer set which may be used in the LAMP method (preferably, the primer set which includes the primers consisting of each of the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 10 to 13, the primer set which includes the primers consisting of each of the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 10 to 15, the primer set which includes the primers consisting of each of the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 16 to 19, or the primer set which includes the primers consisting of each of the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 16 to 21), a DNA polymerase, and dNTPs consisting of dATP,

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dCTP, dGTP, and dTTP. The kit preferably includes the above-mentioned primer set, a variety of oligonucleotides necessary as loop primers (preferably primers consisting of the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 7 and 8), four types of dNTPs serving as substrates of nucleic acid synthesis (dATP, dCTP, dGTP, and dTTP), a DNA polymerase such as a template-dependent nucleic acid synthetase having strand displacement activity, a buffer which provides preferred conditions for enzymatic reactions, a salt serving as a cofactor (such as a magnesium salt or a manganese salt), and a protecting agent for stabilizing an enzyme or a template, and if necessary, reagents necessary for detection of reaction products. The kit of the present invention may include a positive control for confirming whether the LAMP reaction proceeds normally by the primers of the present invention. The positive control is, for example, DNA including a region which is amplified by the method of the present invention.

Further, the kit for detecting *Paecilomyces variotii* of the present invention includes, as nucleic acid primers, an oligonucleotide pair or an oligonucleotide group, which includes at least one pair or one group selected from the group consisting of a pair of the oligonucleotides (c) and (d), a pair of the oligonucleotides (e) and (f), a pair of the oligonucleotides (e) and (g), or a group of the oligonucleotides (e), (f) and (g). The kit can be preferably used in the method of detecting *Paecilomyces variotii* by the PCR method. The kit of the present invention may include not only the above-mentioned nucleic acid primers but also, depending on purpose, substances which are usually used for detecting a fungus, such as a label-detecting substance, a buffer, a nucleic acid synthetase (such as a DNA polymerase, an RNA polymerase, or a reverse transcriptase), and an enzyme substrate (such as dNTP or rNTP). The kit of the present invention may include a positive control for confirming whether the PCR reaction proceeds normally by the primers of the present invention. The positive control is, for example, DNA including a region which is amplified by the method of the present invention.

According to the method of the present invention, a procedure from a sample preparation step to a fungus detection step can be performed within a time as short as about 60 to 120 minutes.

EXAMPLES

Hereinafter, the present invention will be described more in detail with reference to Examples, but it should be understood that the technological scope of the present invention is not particularly limited by the following Examples.

Example 1

Detection of *Paecilomyces variotii* by PCR Method

(1) Analysis of Target Gene

The nucleotide sequence of the β -tubulin gene of *Paecilomyces variotii* was determined by the following method.

A test fungus was cultured in the dark on a potato dextrose agar slant at 25° C. for 7 days. DNA was extracted from the fungus using GenTorukun™ (manufactured by TAKARA BIO INC.). PCR amplification of a target site was performed using PuRe Taq™ Ready-To-Go PCR Beads (manufactured by GE Health Care UK LTD) and, as primers, Bt2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3', SEQ ID NO: 34) and Bt2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3', SEQ ID NO: 35) (Glass and Donaldson, Appl Environ Microbiol 61:1323-1330, 1995). Amplification of β -tubulin partial

length was performed under conditions including a denaturation temperature of 95° C., an annealing temperature of 59° C., an elongation temperature of 72° C., and 35 cycles. PCR products were purified using Auto Seg™ G-50 (manufactured by Amersham Pharmacia Biotech). The PCR products were labeled with BigDye (registered trademark) terminator Ver. 1.1 (manufactured by Applied Biosystems), and electrophoresis was performed using ABI PRISM 3130 Genetic Analyzer (manufactured by Applied Biosystems). Nucleotide sequences from fluorescence signals in electrophoresis were determined using the software "ATGC Ver. 4" (manufactured by Genetyx).

(2) Preparation of Primers

Based on the β -tubulin sequence of *Paecilomyces variotii* determined as above, the β -tubulin sequences of *Hamigera avellanea*, *Talaromyces flavus*, *Talaromyces luteus*, *Talaromyces trachyspermus*, *Byssoschlamys nivea* and *Byssoschlamys fulva* determined in the same way as above, and known β -tubulin sequences of a variety of fungi, alignment analyses were performed using DNA analysis software (Clustal W), to thereby determine nucleotide sequences specific to *Paecilomyces variotii* (SEQ ID NOS: 1 to 4 and 22 to 33). From regions having particularly high specificity to *Paecilomyces variotii* on the 3'-end side in the determined regions, partial regions which satisfy the following four conditions were searched:

- 1) the partial region includes several nucleotides specific to the species;
 - 2) the partial region has a GC content of about 30% to 80%;
 - 3) the partial region has low possibility to cause self-annealing; and
 - 4) the partial region has a Tm value of about 55 to 65° C.
- Based on the nucleotide sequences, five primer pairs including primers which consist of each of the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 5 and 6 were designed. Then, the primers were synthesized by Sigma-Aldrich Japan (desalted products, 0.02 μ mol scale) and purchased.

(3) Preparation of Samples

The fungi shown in Tables 1 and 2 were used as fungi to be used for evaluation of the effectiveness of the designed primers, i.e., *Paecilomyces variotii*, other heat-resistant fungi, and general fungi. These fungi were stored in Medical Mycology Research Center (MMRC), Chiba University, and the fungi deposited based on IFM numbers or the like were obtained and used.

The respective fungi were cultured under optimum conditions. The culture was performed using a potato dextrose medium (trade name: Pearlcore potato dextrose agar medium, manufactured by Eiken Chemical Co., Ltd.) under culture conditions of 25° C. (for general fungi) or 30° C. (for heat-resistant fungi) for 7 days.

TABLE 1

Sample No.	Scientific name	Strain No. (IFM)
1	<i>Paecilomyces variotii</i>	IFM40913
2	<i>Paecilomyces variotii</i>	IFM40915
3	<i>Byssoschlamys flava</i>	IFM48421
4	<i>Byssoschlamys flava</i>	IFM51213
5	<i>Byssoschlamys nivea</i>	IFM51244
6	<i>Byssoschlamys nivea</i>	IFM51245
7	<i>Hamigera avellanea</i>	IFM42323
8	<i>Hamigera avellanea</i>	IFM52241
9	<i>Talaromyces flavus</i>	IFM42243
10	<i>Talaromyces flavus</i>	IFM52233
11	<i>Talaromyces luteus</i>	IFM53242
12	<i>Talaromyces luteus</i>	IFM53241

TABLE 1-continued

Sample No.	Scientific name	Strain No. (IFM)
13	<i>Talaromyces trachyspermus</i>	IFM42247
14	<i>Talaromyces trachyspermus</i>	IFM52252
15	<i>Talaromyces wortmannii</i>	IFM52255
16	<i>Talaromyces wortmannii</i>	IFM52262

TABLE 2

Sample No.	Scientific name	Strain No. (IFM)
1	<i>Paecilomyces variotii</i>	IFM40913
2	<i>Paecilomyces variotii</i>	IFM40915
3	<i>Byssoschlamys nivea</i>	IFM50292
4	<i>Neosartorya fischeri</i>	IFM46945
5	<i>Neosartorya spinosa</i>	IFM46967
6	<i>Neosartorya glabra</i>	IFM46949
7	<i>Neosartorya hiratsukae</i>	IFM47036
8	<i>Aspergillus fumigatus</i>	A125
9	<i>Aspergillus niger</i>	An25
10	<i>Aspergillus terreus</i>	A229
11	<i>Aspergillus flavus</i>	As17
12	<i>Emmericella nidulans</i>	As18
13	<i>Penicillium griseofulvum</i>	IFM54313
14	<i>Penicillium citrinum</i>	IFM54314
15	<i>Alternaria alternata</i>	IFM41348
16	<i>Aureobasidium pullulans</i>	IFM41409
17	<i>Chaetomium globosum</i>	IFM40869
18	<i>Fusarium oxysporum</i>	IFM50002
19	<i>Trichoderma viride</i>	IFM40938
20	<i>Cladosporium cradosporioides</i>	IFM41450

(4) Preparation of Genomic DNA

The respective fungi were collected from the agar media using platinum loops.

Genomic DNA solutions were prepared from the collected fungi using a genomic DNA preparation kit (PrepMan ultra (trade name) manufactured by Applied Biosystems). The concentration of each DNA solution was adjusted to 50 ng/ μ L.

(5) PCR Reaction

One μ L of the genomic DNA solution prepared above as a DNA template, 13 μ L of Pre Mix Taq (trade name, manufactured by TAKARA BIO INC.) and 10 μ L of sterile distilled water were mixed, and 0.5 μ L of a forward primer (20 pmol/ μ L) such as a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 5 (Pae1F primer) and 0.5 μ L of a reverse primer (20 pmol/ μ L) such as a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 6 (Pae1R primer) were added thereto, to thereby prepare 25 μ L of a PCR reaction solution.

The PCR reaction solution was subjected to a gene amplification treatment using an automatic gene amplification device thermal cycler DICE (TAKARA BIO INC.). PCR reaction conditions were 30 cycles of (i) a thermal denaturation reaction at 98° C. for 10 seconds, (ii) an annealing reaction at 63° C. for 1 minute, and (iii) an elongation reaction at 72° C. for 1 minute.

(6) Confirmation of Amplified Gene Fragment

After the PCR reaction, 10 μ L of a sample was collected from the PCR reaction solution and electrophoresed using a 2% agarose gel, and DNA was stained with SYBR Safe DNA gel stain in 1 \times TAE (Invitrogen), followed by fluorescence detection under ultraviolet light, to thereby confirm whether the amplified DNA fragment was present or not. As a result, in the case of using the primer pair including the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 5 and 6 of the designed five primer sets, in a

sample containing genomic DNA of *Paecilomyces variotii*, amplification of gene fragments was confirmed at a position corresponding to the size expected from the designed primer pairs. On the other hand, in a sample containing no genomic DNA of *Paecilomyces variotii*, amplification of gene fragments was not confirmed. FIG. 3 and FIG. 4 show an electrophoretogram in the agarose gel in the case of using the primer pair including the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 5 and 6. Note that, FIG. 3 shows an electrophoretogram of samples of the fungi shown in Table 1, and FIG. 4 shows an electrophoretogram of samples of the fungi shown in Table 2. The numbers in the electrophoretograms represent samples which were subjected to reactions using DNAs extracted from samples of the corresponding sample numbers in the tables.

As a result, in the case of the samples containing the genomic DNA of *Paecilomyces variotii*, amplification of gene fragments of about 250 bp was confirmed. On the other hand, in the case of the samples containing no genomic DNA of *Paecilomyces variotii*, amplification of gene fragments was not confirmed.

The above-described results reveal that *Paecilomyces variotii* can be specifically detected by using the oligonucleotides of the present invention set forth in SEQ ID NOS: 5 and 6. Moreover, the results reveal that it is possible to easily design oligonucleotides for detecting *Paecilomyces variotii*, which can act as nucleic acid probes or nucleic acid primers for specifically detecting *Paecilomyces variotii*, based on the nucleotide sequence of the variable region of the β -tubulin gene of the present invention.

Example 2

Detection of *Paecilomyces variotii* by LAMP Method

(1) Design and Synthesis of Primers

Based on the nucleotide sequence regions which were specified in Example 1 above and specific to *Paecilomyces variotii* (SEQ ID NOS: 1 to 4 and 22 to 33), primers consisting of each of oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 10 to 15 were designed, and the primers were synthesized by E Genome order (FUJITSU SYSTEM SOLUTIONS LIMITED, SEQ ID NOS: 10 and 11; 5 μ mol scale, SEQ ID NOS: 12 and 13; 40 μ mol scale, SEQ ID NOS: 14 and 15; 20 μ mol scale; all of the primers are column-purified products) and purchased.

(2) Preparation of Samples

The fungi shown in Table 3 were used as fungi to be used for evaluation of the effectiveness of the designed primers, i.e., *Paecilomyces variotii* and other heat-resistant fungi. These fungi were stored in Medical Mycology Research Center (MMRC), Chiba University, and the fungi deposited based on IFM numbers or the like were obtained and used.

The respective fungi were cultured under optimum conditions. The culture was performed using a potato dextrose medium (trade name: Pearlcore potato dextrose agar medium, manufactured by Eiken Chemical Co., Ltd.) under culture conditions of 30° C. for 7 days.

TABLE 3

Sample No.	Scientific name	Strain No. (IFM)
1	<i>Paecilomyces variotii</i>	IFM40913
2	<i>Paecilomyces variotii</i>	IFM40915
3	<i>Byssoschlamys flava</i>	IFM48421

TABLE 3-continued

Sample No.	Scientific name	Strain No. (IFM)
4	<i>Byssoschlamys nivea</i>	IFM51244
5	<i>Hamigera avellanea</i>	IFM42323
6	<i>Talaromyces flavus</i>	IFM42243
7	<i>Talaromyces luteus</i>	IFM53242
8	<i>Talaromyces wortmannii</i>	IFM52255

(3) Preparation of Genomic DNA

The respective fungi were collected from the agar media using platinum loops.

Genomic DNA solutions were prepared from the collected fungi using a genomic DNA preparation kit (PrepMan ultra (trade name) manufactured by Applied Biosystems). Specifically, several colonies were collected from each medium, and the fungus was suspended in 200 μ L of a reagent supplied with the kit and dissolved by a heat treatment at 100° C. for 10 minutes. Centrifugation was performed at 14,800 rpm for 5 minutes, and the supernatant was collected. The concentration of the resultant genomic DNA solution was adjusted to 50 ng/ μ L. The genomic DNA solution was used as a template DNA in the following LAMP reaction.

(4) Preparation of Reaction Solution for LAMP Reaction

12.5 μ L of 2 \times Reaction Mix (Tris-HCl (pH 8.8) 40 mM, KCl 20 mM, MgSO₄ 16 mM, (NH₄)₂SO₄ 20 mM, 0.2% Tween20, Betaine 1.6 M, dNTPs 2.8 mM: Eiken Chemical Co., Ltd.; Loopamp DNA amplification reagent kit), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 10 (LPae1F3 primer: 5 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 11 (LPae1B3 primer: 5 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 12 (LPae1FIP primer: 40 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 13 (LPae1BIP primer: 40 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 14 (LPae1LF loop primer: 20 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: (LPae1LB loop primer: 20 pmol/ μ L), 1 μ L of Bst DNA Polymerase (8 U/25 μ L, manufactured by Eiken Chemical Co., Ltd.) and 1 μ L of the template DNA prepared above were mixed, and distilled water was added thereto, to thereby prepare a total of 25 μ L of a reaction solution.

(5) LAMP Reaction

The reaction solution prepared above was subjected to a DNA amplification reaction at 63 \pm 2° C. for 60 minutes using a real-time turbidity measuring apparatus Loopamp RT-160C (manufactured by Eiken Chemical Co., Ltd.). Simultaneously, the turbidity of the reaction solution was measured (wavelength: 400 nm).

(6) Confirmation of DNA Amplification

Amplification of DNA was judged to be present or not by whether or not the turbidity increased in the reaction solution. The measurement results of the turbidity of the reaction solutions are shown in FIG. 5.

As a result, the turbidity increases, that is, the DNA synthesis and amplification reactions were observed from about 23 minutes after the initiation of the reaction only in the systems where the genomic DNAs of *Paecilomyces variotii* were used as templates.

On the other hand, in the systems where the genomic DNAs of other fungi were used, the turbidity increases in the reac-

tion solutions were not observed for 40 minutes after the initiation of the reaction. Note that, also in the systems where the genomic DNAs of fungi other than *Paecilomyces variotii* were used, the turbidity increases of the reaction solutions were observed from about 45 minutes after the initiation of the reaction. However, the phenomena are probably caused by time-dependent occurrence of a nonspecific reaction among primers or by a reaction of a small amount of primers to sites other than the target due to a long reaction time.

Example 3

Detection of *Paecilomyces variotii* by LAMP Method

(1) Design and Synthesis of Primers

Based on the nucleotide sequence regions which were specified in Example 1 above and specific to *Paecilomyces variotii* (SEQ ID NOS: 1 to 4 and 22 to 33), primers consisting of each of oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 16 to 21 were designed, and the primers were synthesized by E Genome order (FUJITSU SYSTEM SOLUTIONS LIMITED, SEQ ID NOS: 16 and 17: 5 μ mol scale, SEQ ID NOS: 18 and 19: 40 pmol scale, SEQ ID NOS: 20 and 21: 20 μ mol scale; all of the primers are column-purified products) and purchased.

(2) Preparation of Samples

The fungi shown in Table 4 were used as fungi to be used for evaluation of the effectiveness of the designed primers, i.e., *Paecilomyces variotii*, other heat-resistant fungi, and general fungi. These fungi were stored in Medical Mycology Research Center (MMRC), Chiba University, and the fungi deposited based on IFM numbers or the like were obtained and used.

The respective fungi were cultured under optimum conditions. The culture was performed using a potato dextrose medium (trade name: Pearlcore potato dextrose agar medium, manufactured by Eiken Chemical Co., Ltd.) under culture conditions of 25° C. (for general fungi) or 30° C. (for heat-resistant fungi) for 7 days.

TABLE 4

Sample No.	Scientific name	Strain No. (IFM)
1	<i>Paecilomyces variotii</i>	IFM40913
2	<i>Paecilomyces variotii</i>	IFM40915
3	<i>Byssosclamyces flava</i>	IFM48421
4	<i>Hamigera avellanea</i>	IFM42323
5	<i>Penicillium griseofulvum</i>	IFM54313
6	<i>Penicillium citrinum</i>	IFM54314
7	<i>Neosartorya fischeri</i>	IFM46945
8	<i>Neosartorya spinosa</i>	IFM46967
9	<i>Neosartorya glabra</i>	IFM46949
10	<i>Neosartorya hiratsukae</i>	IFM47036
11	<i>Altieraria alternate</i>	IFM41348
12	<i>Aureobasidium pullulans</i>	IFM41409
13	<i>Chaetomium globosum</i>	IFM40869
14	<i>Fusarium oxysporum</i>	IFM50002
15	<i>Trichoderma viride</i>	IFM40938
16	<i>Cladosporium cradosporioides</i>	IFM41450

(3) Preparation of Genomic DNA

The respective fungi were collected from the agar media using platinum loops.

Genomic DNA solutions were prepared from the collected fungi using a genomic DNA preparation kit (PrepMan ultra (trade name) manufactured by Applied Biosystems). Specifically, several colonies were collected from each medium, and the fungus was suspended in 200 μ L of a reagent supplied

with the kit and dissolved by a heat treatment at 100° C. for 10 minutes. Centrifugation was performed at 14,800 rpm for 5 minutes, and the supernatant was collected. The concentration of the resultant genomic DNA solution was adjusted to 50 ng/ μ L. The genomic DNA solution was used as a template DNA in the following LAMP reaction.

(4) Preparation of Reaction Solution for LAMP Reaction

12.5 μ L of 2 \times Reaction Mix (Tris-HCl (pH 8.8) 40 mM, KCl 20 mM, MgSO₄ 16 mM, (NH₄)₂SO₄ 20 mM, 0.2% Tween20, Betaine 1.6 M, dNTPs 2.8 mM: Eiken Chemical Co., Ltd.; Loopamp DNA amplification reagent kit), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 16 (LPae2F3 primer: 5 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 17 (LPae2B3 primer: 5 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 18 (LPae2FIP primer: 40 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 19 (LPae2BIP primer: 40 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: (LPae2LF loop primer: 20 pmol/ μ L), 1 μ L of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 21 (LPae2LB loop primer: 20 pmol/ μ L), 1 μ L of Bot DNA Polymerase (8 U/25 μ L, manufactured by Eiken Chemical Co., Ltd.) and 1 μ L of the template DNA prepared above were mixed, and distilled water was added thereto, to thereby prepare a total of 25 μ L of a reaction solution.

(5) LAMP Reaction

The reaction solution prepared above was subjected to a DNA amplification reaction at 63 \pm 2° C. for 60 minutes using a real-time turbidity measuring apparatus Loopamp RT-160C (manufactured by Eiken Chemical Co., Ltd.). Simultaneously, the turbidity of the reaction solution was measured (wavelength: 400 nm).

(6) Confirmation of DNA Amplification

Amplification of DNA was judged to be present or not by whether or not the turbidity increased in the reaction solution. The measurement results of the turbidity of the reaction solutions are shown in FIG. 6.

As a result, the turbidity increases, that is, the DNA synthesis and amplification reactions were observed from about 20 minutes after the initiation of the reaction only in the systems where the genomic DNAs of *Paecilomyces variotii* were used as templates.

On the other hand, in the systems where the genomic DNAs of other fungi were used, the turbidity increases in the reaction solutions were not observed for 50 minutes after the initiation of the reaction. Note that, also in the systems where the genomic DNAs of fungi other than *Paecilomyces variotii* were used, the turbidity increases of the reaction solutions were observed from about 60 minutes after the initiation of the reaction. However, the phenomena are probably caused by time-dependent occurrence of a nonspecific reaction among primers or by a reaction of a small amount of primers to sites other than the target due to a long reaction time.

Example 4

Detection of *Paecilomyces variotii* by PCR Method

(1) Preparation of Primers

From the nucleotide sequence regions which were specified in Example 1 above and specific to *Paecilomyces variotii*

(SEQ ID NOS: 1 to 4 and 22 to 33), partial regions which satisfy the following four conditions were searched:

- 1) the partial region includes several nucleotides specific to the species;
- 2) the partial region has a GC content of about 30% to 80%;
- 3) the partial region has low possibility to cause self-annealing; and
- 4) the partial region has a T_m value of about 55 to 65° C.

Based on the nucleotide sequences, five primer pairs including primers which consist of each of the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 7 to 9 were designed. Then, the primers were synthesized by Sigma-Aldrich Japan (desalted products, 0.02 μmol scale) and purchased.

(2) Preparation of Samples

The fungi shown in Table 5 were used as fungi to be used for evaluation of the effectiveness of the designed primers, i.e., *Paecilomyces variotii* and other heat-resistant fungi. These fungi were stored in Medical Mycology Research Center (MMRC), Chiba University, and the fungi deposited based on IFM numbers or the like were obtained and used.

The respective fungi were cultured under optimum conditions. The culture was performed using a potato dextrose medium (trade name: Pearlcore potato dextrose agar medium, manufactured by Eiken Chemical Co., Ltd.) under culture conditions of 30° C. for 7 days.

TABLE 5

No.	Strain	scientific name
1	NBRC4855	<i>Pae. variotii</i>
2	NBRC7563	<i>Pae. variotii</i>
3	NBRC5479	<i>Pae. variotii</i>
4	NBRC31685	<i>Pae. variotii</i>
5	IFM48421	<i>B. fluva</i>
6	IFM51213	<i>B. fluva</i>
7	IFM51244	<i>B. nivea</i>
8	IFM51245	<i>B. nivea</i>
9	IFM42323	<i>H. avellanea</i>
10	IFM52241	<i>H. avellanea</i>
11	IFM42243	<i>T. flavus</i>
12	IFM53242	<i>T. luteus</i>
13	IFM42247	<i>T. trachyspermum</i>
14	IFM52255	<i>T. wortmannii</i>
15	IFM53622(T)	<i>T. ebumeus</i>

(3) Preparation of Genomic DNA

The respective fungi were collected from the agar media using platinum loops.

Genomic DNA solutions were prepared from the collected fungi using a genomic DNA preparation kit (PrepMan ultra (trade name) manufactured by Applied Biosystems). The concentration of each DNA solution was adjusted to 50 ng/μL.

(4) PCR Reaction

One μL of the genomic DNA solution prepared above as a DNA template, 15 μL of Pre Mix Taq (trade name, manufactured by TAKARA BIO INC.) and 11 μL of sterile distilled water were mixed, and 1 μL of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 7 (Pae4F primer, 20 pmol/μL), 1 μL of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 8 (Pae4R-1 primer, 20 pmol/μL) and 1 μL of a primer consisting of an oligonucleotide represented by the nucleotide sequence set forth in SEQ ID NO: 9 (Pae1R-2 primer, 20 pmol/μL) were added thereto, to thereby prepare 30 μL of a PCR reaction

solution. PCR reaction solutions for the other four primer pairs were prepared in the similar manner described above.

The PCR reaction solution was subjected to a gene amplification treatment using an automatic gene amplification device thermal cycler DICE (TAKARA BIO INC.). PCR reaction conditions were 35 cycles of (i) a thermal denaturation reaction at 95° C. for 1 minute, (ii) an annealing reaction at 59° C. for 1 minute, and (iii) an elongation reaction at 72° C. for 1 minute.

(5) Confirmation of Amplified Gene Fragment

After the PCR reaction, 2.5 μL of a sample was collected from the PCR reaction solution and electrophoresed using a 2% agarose gel, and DNA was stained with SYBR Safe DNA gel stain in 1×TAE (Invitrogen), followed by fluorescence detection under ultraviolet light, to thereby confirm whether the amplified DNA fragment was present or not. As a result, in the case of using the primer pair consisting of the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 7 to 9 of the designed five primer sets, in a sample containing genomic DNA of *Paecilomyces variotii*, amplification of gene fragments was confirmed at a position corresponding to the size expected from the designed primer pairs. On the other hand, in a sample containing no genomic DNA of *Paecilomyces variotii*, amplification of gene fragments was not confirmed. FIG. 7 shows an electrophoretogram in the agarose gel in the case of using the primer set including the oligonucleotides represented by the nucleotide sequences set forth in SEQ ID NOS: 7 to 9. The numbers in the electrophoretograms represent samples which were subjected to reactions using DNAs extracted from samples of the corresponding sample numbers in Table 5.

As a result, in the case of the samples containing the genomic DNA of *Paecilomyces variotii*, amplification of gene fragments of about 200 bp was confirmed. On the other hand, in the case of the samples containing no genomic DNA of *Paecilomyces variotii*, amplification of gene fragments was not confirmed.

The above-described results reveal that *Paecilomyces variotii* can be specifically detected by using the oligonucleotides of the present invention. Moreover, the results reveal that it is possible to easily design oligonucleotides for detecting *Paecilomyces variotii*, which can act as nucleic acid probes or nucleic acid primers for specifically detecting *Paecilomyces variotii*, based on the nucleotide sequence of the variable region of the β-tubulin gene of the present invention.

As is clear from the results of Examples 1 to 4, according to the method of the present invention, it is possible to detect *Paecilomyces variotii* easily, rapidly, and specifically.

Industrial Applicability

According to the method of detecting *Paecilomyces variotii* of the present invention, it is possible to provide a method with which *Paecilomyces variotii* can be detected specifically, easily, and rapidly. Therefore, the method of detecting *Paecilomyces variotii* of the present invention is useful for detection of *Paecilomyces variotii*, which is known to be a harmful fungus in the food industry, the toiletry industry, and the like.

Having described our invention as related to the present embodiments, it is our intention that the invention not be limited by any of the details of the description, unless otherwise specified, but rather be construed broadly within its spirit and scope as set out in the accompanying claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 35

<210> SEQ ID NO 1

<211> LENGTH: 513

<212> TYPE: DNA

<213> ORGANISM: *Paecilomyces variotii*

<220> FEATURE:

<223> OTHER INFORMATION: strain IMF40913

<400> SEQUENCE: 1

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tggttaaccaa atcgggtgctg ctttctggta tgttgtaaac cagcaggaga aatgaaacaa      60
agagcctaga gtccgtttgg ggacgtggaa ggctcaagtg atcagaattg gaggtgctaa      120
cgatcctata ggcagaccaa ctctggtgag caggccttg acggctctgg tgtgtaagta      180
cacgatatct ggggatgctt cgacacggaa tcgagagaga cgactgacga tggattagct      240
acaatggctc ctccgacctt cagctcgagc gcatgaacgt ctacttcaac gaggtagtgt      300
ttgacctat gatcccaaga ggaacgctcc atgagctcac caataaatag gccgctggca      360
agaagtacgt tcctcgctgc gtccctcgctg acctcgagcc tggtaaccatg gacgctgtcc      420
gtgctggctc tttcgccag ctcttcgcc ctgacaactt cgtcttcggt cagtcgggtg      480
ctggttaacaa ctgggccaag ggtcactaca ctg                                     513

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<210> SEQ ID NO 2

<211> LENGTH: 515

<212> TYPE: DNA

<213> ORGANISM: *Paecilomyces variotii*

<220> FEATURE:

<223> OTHER INFORMATION: strain IMF40915

<220> FEATURE:

<221> NAME/KEY: misc_feature

<222> LOCATION: (258)..(258)

<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 2

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agagcctgga gtccgtttgg ggacgtcgaa ggctcaagtg atgagacttg gaggtgctaa      120
cgaccctata ggcagaccaa ctctggtgag caggccttg acggctccgg tgtgtaagta      180
cacgatatct ggggatgctt cgacacggaa tcgagagaga cgactgacga tggattagct      240
acaatggctc ctccgacnac cttcagctcg agcgcatgaa tgtctacttc aacgaggtag      300
ttgttgacct tatgatccca agaggaacgc cccatgagct caccaataaa taggcgctg      360
gcaagaagta cgttctcgt gccgtcctcg tcgacctcga gcctggtaacc atggacgctg      420
tccgtgctgg tcctttcggc cagctcttcc gccctgacaa cttcgtcttc ggtcagtcgg      480
gtgctggtaa caactgggcc aaggtcacta cactg                                     515

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<210> SEQ ID NO 3

<211> LENGTH: 518

<212> TYPE: DNA

<213> ORGANISM: *Paecilomyces variotii*

<220> FEATURE:

<223> OTHER INFORMATION: strain EU037073

<400> SEQUENCE: 3

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ggtaacacaaa tcggtgctgc tttctggtat gttggaaatc aataggagaa atgaaagaaa      60
gagcccggtg gtcatattga gacgtggaag gctcagggtta tgaagattga ggggtgctaac      120
tatgctatag gcagaccatc tctggcgagc acggcattga cggctctggt gtgtgagtac      180
acacgatatc tggagacgct tggacatcga atcgacgggt gactgacgat ggattagcta      240

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caatggcacc tccgacctcc agctcgagcg catgaacgtc tacttcaacg aggtagttgt	300
tggccctatg ataccgataa ggacgctcca tatgctcacc aatactttag gctgctggca	360
agaagtatgt tcctcgtgcc gtctcgtcg accttgagcc tggtagcatg gacgctgtcc	420
cgtgccggtc ctttcggcca gctcttcgc cctgacaact tcgtcttcgg tcagtcgccg	480
tgccgtaaca actgggcca gggtcactac actgaggg	518

<210> SEQ ID NO 4
 <211> LENGTH: 519
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain IFM50293

<400> SEQUENCE: 4

tggtaaccaa atcgggtgctg ctttctggta tgttgaccag caggagaaat gaaacaacga	60
gcctggggtc catttgggga cgtggaaggc tcagatgatg agaattggag gtgctaacga	120
tcctacaggc agaccatctc tggcgagcac ggccttgacg gctctggtgt gtaagtacac	180
acgatatctc gaggcgtttc gacgtggaat cgagagagat gactgacgat gaattagcta	240
caatggctcc tccgaccttc agctcgagcg catgaacgtc tacttcaacg aggtagttgt	300
tgaccctatg atcccaaaag gaacgcccc tgagctcacc aataaatagg ccgctggcaa	360
gaagtacgtc cctcgtgccg tcctcgtcga cctcgagcct ggtaccatgg acgctgtccg	420
tgccggctct ttcggccagc tcttcgcccc tgacaacttc gtcttcggtc agtcgggtgc	480
tggtaaccaac tgggccaagg gtcactacac tgagggtag	519

<210> SEQ ID NO 5
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 5

cctagagtcc gtttggggac	20
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<210> SEQ ID NO 6
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 6

gttcctcttg ggatcatagg	20
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<210> SEQ ID NO 7
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 7

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<210> SEQ ID NO 8
 <211> LENGTH: 20
 <212> TYPE: DNA

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<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 8

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<210> SEQ ID NO 9
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 9

gctcatgggg cgctcctctt                20

<210> SEQ ID NO 10
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 10

acgatacctat aggcagacca                20

<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 11

ccagcggcct atttattggt                20

<210> SEQ ID NO 12
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 12

cgtctctctc gattccgtgt cgccttgacg gctctgggtg                40

<210> SEQ ID NO 13
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 13

ctccgacctt cagctcgagc ggagcggttc tcttgggat                39

<210> SEQ ID NO 14
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 14

tccccagata tcgtgtactt ac                22

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<210> SEQ ID NO 15
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 15

acttcaacga ggtagttggt g 21

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 16

cgatatctgg ggatgcttcg 20

<210> SEQ ID NO 17
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 17

cgtccatggt accaggct 18

<210> SEQ ID NO 18
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 18

atgcgctcga gctgaagggtc cggaatcgag agagagacga ct 42

<210> SEQ ID NO 19
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 19

tgatcccaag aggaacgccc cacgaggaac gtacttcttg cc 42

<210> SEQ ID NO 20
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 20

ggaggagcca ttgtagctaa 20

<210> SEQ ID NO 21
<211> LENGTH: 21
<212> TYPE: DNA
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<220> FEATURE:

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<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 21

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<210> SEQ ID NO 22

<211> LENGTH: 470

<212> TYPE: DNA

<213> ORGANISM: Paecilomyces variotii

<220> FEATURE:

<223> OTHER INFORMATION: strain NBRC4855

<400> SEQUENCE: 22

gatgaaagaa agagccccggg tgcatattg agacgtggaa ggctcagggt atgaagattg 60

aggggtgctaa ctatgctata ggcagaccat ctctggcgag caccgccttg acggctctgg 120

tgtgtgagta cacacgatat ctggagacgc ttggacatcg aatcgacggg tgactgacga 180

tggattagct acaatggcac ctccgacctc cagctcgagc gcatgaacgt ctactcaac 240

gaggtagttg ttggccctat gataccgata aggacgtcc atatgctcac caatacttta 300

ggctgctggc aagaagtatg ttctcgtgc cgtcctcgtc gaccttgagc ctggtaccat 360

ggacgctgtc cgtgccggtc ctttcggcca gctcttcgc cctgacaact tcgtcttcgg 420

tcagtcgggt gctggtaaca actgggcca gggtcactac actgagggtg 470

<210> SEQ ID NO 23

<211> LENGTH: 506

<212> TYPE: DNA

<213> ORGANISM: Paecilomyces variotii

<220> FEATURE:

<223> OTHER INFORMATION: strain IFM52147

<400> SEQUENCE: 23

aaatcgggtg tgctttctgg tatgttcgaa atcaatagga gaaatgaaag aaagagcccg 60

gggtgcatcc tgagacgtgg aaggctcagg ttatgaagat tgagggtgct aactatgcta 120

taggcagacc atctctggcg agcacggcct tgacggctct ggtgtgtgag tacacacgat 180

atctggagac gcttggacat cgaatcaacg ggtgactgac gatggattag ctacaatggc 240

acctccgacc tccagctcga gcgcataaac gtctacttca atgaggtagt tgttggccct 300

atgataccga taaggacgct ccatatgctc accaatactt taggctgctg gcaagaagta 360

tgttctcgt gccgtactcg tcgacctga gcccggtacc atggacgctg tccgtgccgg 420

tccttccggc cagctcttcc gccctgacaa ctctgtcttc ggtcagtcgg gtgctggtaa 480

caactggggc aagggtcact acactg 506

<210> SEQ ID NO 24

<211> LENGTH: 518

<212> TYPE: DNA

<213> ORGANISM: Paecilomyces variotii

<220> FEATURE:

<223> OTHER INFORMATION: strain IFM52145

<400> SEQUENCE: 24

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aagagccccg gtgtctgttt gagacgtgga aggtcagggt tatgaagatt gaggggtgcta 120

attatgctat aggagaccca tctctggcga gcacggcctt gacgggtctg gtgtgtgagt 180

acacgatatc tggagacgct tggacatcga atcgacgggt gactgacgat ggattagcta 240

caatggcacc tccgacctcc agctcgagcg catgaacgtc tacttcaacg aggtagtgtg 300

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tggccctatg ataccgataa ggacgctcca tatgctcacc aatactttag gctgctggca	360
agaagtatgt tctcgtgccc gtcctcgtcg accttgagcc tggtagcatg gacgctgtcc	420
gtgccgggtcc ttctggccag ctcttcgcc ctgacaactt cgtcttcggt cagtcgggtg	480
ctggtaacaa ctgggccaag ggtcactaca ctgagggt	518

<210> SEQ ID NO 25
 <211> LENGTH: 461
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain IFM51028

<400> SEQUENCE: 25

aatgaaaga aagagcccg gtgtctgttt gagacgtgga aggtcaggt tatgaagatt	60
gaggggtgcta actatgctat aggcagacca tctctggcga gcacggtctt gacggtctg	120
gtgtgtgagt acacacgata tctggagacg cttggacatc gaatcgacgg gtgactgacg	180
atggattagc tacaatggca cctccgacct ccagctcgag cgcataaacg tctacttcaa	240
cgaggtagtt gttggcccta tgatactgat aaggacgctc catatgctca ccaacacttt	300
aggctgctgg caagaagtat gttcctcgtg ccgtcctcgt cgaccttgag cctggtagca	360
tggacgctgt ccgtgccggt cctttcggcc agctcttcgg ccctgacaac ttcgtcttcg	420
gtcagtcagg tgctggtaac aactgggcca agggtcacta c	461

<210> SEQ ID NO 26
 <211> LENGTH: 522
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain IFM51195

<400> SEQUENCE: 26

gtggtaacca aatcgggtgct gctttctggt atgttggaat tcaataggag aatgaaaga	60
aggagcccg gtgtctgttt gaggcgtgga aggtcaggt tatgaagatt gaggggtgcta	120
actatgctat aggcagacca tctctggcga gcacggtctt gacggtctg gtgtgtgagt	180
acacacgata tctggagacg cttggacatc gaatcgacgg gtgactgacg atggattagc	240
tacaatggca cctccgacct ccagctcgag cgcataaacg tctacttcaa cgaggtagtt	300
gttgcccta tgataccgat aaggacgctc catatgctca ccgataaatt aggtgctgg	360
caagaagtat gttccacgtg ccgtcctcgt cgaccttgag cctggtagca tggacgctgt	420
ccgtgccggt cctttcggcc agctcttcgg ccctgacaac ttcgtcttcg gtcagtcagg	480
tgctggtaac aactgggcca agggtcacta cactgagggt ag	522

<210> SEQ ID NO 27
 <211> LENGTH: 523
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain IFM55619

<400> SEQUENCE: 27

agtggtaacc aatcgggtgc tgctttctgg tatgttgga atcaagagga gaaatgaaag	60
aaagagcccg ggtgtctgtt tgagacgtgg aaggctcagg ttatgaagat tgagggtgct	120
aactatgcta taggcagacc atctctggcg agcacggtct tgacggctct ggtgtgtgag	180

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tacacacgat atctggagac gcttggacat cgaatcgacg ggtgactgac gatggattag	240
ctacaatggc acctccgacc tccagctcga gcgcatgaac gtctacttca acgaggtagt	300
tgttggccct atgatactga taaggacgct ccatatgctc accaactctt taggtgctg	360
gcaagaagta tgttcctcgt gccgtcctcg tcgacctga gccctggacc atggacgctg	420
tccgtgccgg tcccttcggc cagctcttcc gccctgacaa ctctgtcttc ggtcagtcgg	480
gtgctggtaa caactggggc aagggtcact acactgaggg tag	523

<210> SEQ ID NO 28
 <211> LENGTH: 463
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain NBRC5479

<400> SEQUENCE: 28

acaacgagcc tgggggtccat ttgagggcgt ggaaggctca gatgatgaga attgaagggtg	60
ctaacgatcc tacaggcaga ccatctctgg cgagcacggc cttgacggct ctggtgtgta	120
agtacacacg atatctcaga gcacttcgac gcggaatcga gagagaactg acgatggatt	180
agctacaatg gctcttcoga ccttcagctc gagcgcatga acgtctactt caacgaggta	240
gttggtgacc ctatgatccc aagaggaacg ccccatgagc tcaccaataa ataggccgct	300
ggcaagaagt acgttctctg tgccgtcctc gtcgaccttg agcctggtag catggacgct	360
gtccgcgcgg gtcctttcgg ccagctcttc cgccctgaca actctgtctt cggtcagtcg	420
ggtgctggta acaactgggc caagggtcac tacactgagg gta	463

<210> SEQ ID NO 29
 <211> LENGTH: 518
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain IFM51027

<400> SEQUENCE: 29

ggtaacaaaa tcggtgctgc tttctggtat gttgacaacc agctggagaa atggaacaac	60
gagcctgggg tccatttgag ggtgtggaag gctcagatga tgagaattga aggtgctaac	120
gatcctacag gcagaccatc tctggcgagc acggccttga cggtcttggt gtgtaagtac	180
acccgatatc tcagagcact tcgacgcgga atcgagagag aactgacgat ggattagcta	240
caatggctcc tccgatcttc agctcgagcg catgaacgct tacttcaacg aggtagtgtg	300
tgaccctatg atcccaagag gaacgcccc tgagctcacc aataaatagg ccgctggcaa	360
gaagtacgtt cctcgtgccg tctcgtcga ccttgagcct ggtaccatgg acgctgtccg	420
tgccggctct ttcggccagc tcttccgcc tgacaacttc gtcttcggtc agtcgggtgc	480
tggttaacaac tgggccaagg gtcactacac tgagggta	518

<210> SEQ ID NO 30
 <211> LENGTH: 450
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain NBRC31967

<400> SEQUENCE: 30

aaacaaagag cctggagttc atttggtcgt agtgatcaga attggagggt gctaacgacc	60
ctataggcag accatctctg gtgagcacgg ccttgacggc tctggtgtgt aagtacacga	120

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tatctgggga tgcttcgaca cggaatcgag acgactgacg atggattagc tacaatggct	180
cctccgacct tcagctcgag cgcataaacg tctacttcaa cgaggtagtt gttggcccta	240
taatcccaag aggaacgccc catgagctca ccaataaata ggccgctggc aagaagtacg	300
ttcctcgtgc cgtcctcgtc gacctcgagc ctggtaccat ggacgctgtc cgtgctggtc	360
ctttcggcca gctcttcgc cctgacaact tcgtcttcgg tcagtcgggt gctggtaaca	420
actgggcca gggtcactac actgaagggt	450

<210> SEQ ID NO 31
 <211> LENGTH: 497
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain NBRC31685
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (7)..(7)
 <223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 31

tggtaancaa atcgggtgctg ctttctggta tgttgtcaac cagcaggaga aatgaaacaa	60
agagcctgga gttcatttgg ctgcagtgat cagaattgga ggtgctaacg accctatagg	120
cagaccatct ctggtgagca cggccttgac ggctctgggtg tgtaagtaca cgatatctgg	180
ggatgcttcg acacggaatc gagacgactg acgatggatt agctacaatg gctcctccga	240
ccttcagctc gagecgaatg acgtctactt caacgaggta gttgttgccc ctataatccc	300
aagaggaaacg ccccatgagc tcaccaataa ataggccgct ggcaagaagt acgttcctcg	360
tgccgtcctc gtcgacctcg agcctggtag catggacgct gtccgtgctg gtcctttcgg	420
ccagctcttc cgcctcgaca acttcgtctt cggtcagtcg ggtgctggta acaactgggc	480
caagggtcac tacactg	497

<210> SEQ ID NO 32
 <211> LENGTH: 519
 <212> TYPE: DNA
 <213> ORGANISM: Paecilomyces variotii
 <220> FEATURE:
 <223> OTHER INFORMATION: strain SUM3339
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (1)..(1)
 <223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 32

ntggtaacca aatcggtgct gctttctggt atgttgtcaa ccagcaggag aaatgaaaca	60
aagagcctgg agtccgtttg gggacgtcga aggtcgaagt gatgagaatt ggagggtgcta	120
acgaccctat aggcagacca tctctggtga gcacggcctt gacggctctg gtgtgtaagt	180
acacgatatc tggggatgct tcgacacgga atcgagagag acgactgacg atggattagc	240
tacaatggct cctccgacct tcagctcgag cgcataaacg tctacttcaa cgaggtagtt	300
gttggcccta taatcccaag aggaacgccc catgagctca ccaataaata ggccgctggc	360
aagaagtacg ttcctcgtgc cgtcctcgtc gacctcgagc ctggtaccat ggacgctgtc	420
cgtgctggtc ctttcggcca gctcttcgc cctgacaact tcgtcttcgg tcagtcgggt	480
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<210> SEQ ID NO 33

-continued

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<211> LENGTH: 516
<212> TYPE: DNA
<213> ORGANISM: Paecilomyces variotii
<220> FEATURE:
<223> OTHER INFORMATION: strain IFM50294

<400> SEQUENCE: 33

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cgaccctata ggcagaccaa cttctggtgag caccgccttg acggetcttg tgtgtaagta      180
cacgatatct ggggatgctt cgacacggaa tcgagacgac tgacgatgga ttagctacaa      240
tggtctctcc gaccttcagc tcgagcgcat gaacgtctac ttcaacgagg tagttgttgg      300
ccctataatc ccaagaggaa cgccccatga gctcaccaat aaataggccg ctggcaagaa      360
gtacgttctc cgtgccgtcc tcgtcgacct cgagcctggt accatggacg ctgtccgtgc      420
tggtccttcc ggccagctct tcgcgcctga caacttcgtc ttcggtcagt ccggtgctgg      480
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<210> SEQ ID NO 34
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<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 34

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<210> SEQ ID NO 35
<211> LENGTH: 24
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<400> SEQUENCE: 35

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What is claimed is:

1. A method of detecting whether *Paecilomyces variotii* is present in a sample, said method comprising
 - (I) adding, to said sample, or adding to DNA prepared from DNA or RNA obtained from said sample, a primer pair or primer group selected from the group consisting of:
 - (i) a primer pair in which the nucleotide sequence of one primer is the nucleotide sequence of SEQ ID NO: 5 and the nucleotide sequence of the other primer is the nucleotide sequence of SEQ ID NO: 6;
 - (ii) a primer pair in which the nucleotide sequence of one primer is the nucleotide sequence of SEQ ID NO: 7 and the nucleotide sequence of the other primer is the nucleotide sequence of SEQ ID NO: 8;
 - (iii) a primer pair in which the nucleotide sequence of one primer is the nucleotide sequence of SEQ ID NO: 7 and the nucleotide sequence of the other primer is the nucleotide sequence of SEQ ID NO: 9; and
 - (iv) a primer group of three primers in which the nucleotide sequence of one primer is the nucleotide sequence of SEQ ID NO: 7, the nucleotide sequence of one primer is the nucleotide sequence of SEQ ID NO: 8, and the nucleotide sequence of one primer is the nucleotide sequence of SEQ ID NO: 9;

- under conditions in which the primers in said primer pair or primer group hybridize to *Paecilomyces variotii* nucleic acid if said *Paecilomyces variotii* nucleic acid is present,
 - (II) performing an amplification reaction that amplifies, if present, the *Paecilomyces variotii* nucleic acid of part (I), to which said primers in said primer pair or said primer group are hybridized; and
 - (III) detecting whether *Paecilomyces variotii* nucleic acid was amplified in part (II), wherein detecting *Paecilomyces variotii* nucleic acid that was amplified in part (II) is as an indication of the presence of *Paecilomyces variotii* in said sample.
2. The method of claim 1, wherein the primer pair or primer group is that of part (I)(i), a primer pair in which the nucleotide sequence of one primer is the nucleotide sequence of SEQ ID NO: 5 and the nucleotide sequence of the other primer is the nucleotide sequence of SEQ ID NO: 6.
 3. The method of claim 1, wherein the amplification reaction of part (II) is performed by a polymerase chain reaction (PCR) method.
 4. The method of claim 3, wherein the polymerase chain reaction (PCR) method is performed using the primer pair of part (I)(i), a primer pair in which the nucleotide sequence of

one primer is the nucleotide sequence of SEQ ID NO: 5 and the nucleotide sequence of the other primer is the nucleotide sequence of SEQ ID NO: 6.

5. The method of claim 3, which further comprises electrophoresing or sequencing a product or products of the PCR. 5

6. The method of claim 3, wherein the PCR method integrates a nucleotide labeled with a radioactive substance or a fluorescent substance into a product or products of the PCR.

7. The method of claim 1, wherein said primer pair or said primer group is added to said sample. 10

8. The method of claim 1, wherein said primer pair or said primer group is added to DNA prepared from DNA or RNA obtained from said sample.

9. The method of claim 1, wherein the primer pair or primer group is that of part (I)(ii), (I)(iii) or (I)(iv). 15

10. The method of claim 9, wherein the primer pair or primer group is that of part (I)(ii).

11. The method of claim 9, wherein the primer pair or primer group is that of part (I)(iii).

12. The method of claim 9, wherein the primer pair or primer group is that of part (I)(iv). 20

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